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Positron emission tomography *in vivo* characterisation of the pathology of frontotemporal dementia



**UNIVERSITY OF
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William Richard Bevan-Jones

Peterhouse

April 2019

This thesis is submitted for the degree of Doctor of Philosophy

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Summary

Positron emission tomography *in vivo* characterisation of the pathology of frontotemporal dementia

William Richard Bevan-Jones

Frontotemporal dementia (FTD) is clinically and pathologically diverse, encompassing the behavioural variant FTD; non-fluent variant primary progressive aphasia; and semantic variant primary progressive aphasia. These are usually associated with either tau or TDP-43 pathology, with highly variable clinicopathological correlations. Neuroinflammation also contributes to the pathogenesis of FTD, but its relevance to the disease spectrum is incompletely understood. There is a critical need for better understanding of how drivers of pathophysiology, such as neuroinflammation and protein aggregation, relate to the heterogeneity of clinical disease *in vivo*. This knowledge gap currently forms a significant barrier to the development of effective treatments in FTD.

I review the clinical, pathological and genetic features of FTD and the role of PET for measuring *in vivo* components of pathophysiology in this setting. I then describe a series of case studies and group analysis of FTD syndromes, using positron emission tomography (PET) radioligands to visualise and quantify different aspects of pathophysiology *in vivo*.

[¹⁸F]AV-1451tau was introduced primarily to study tau pathology in Alzheimer's disease using, which differs from FTD tauopathy in several respects. I examined the sensitivity and specificity of [¹⁸F]AV-1451 in FTD, *in vivo*, through (i) [¹⁸F]AV-1451 imaging of the FTLD-tau pathology in a case of FTD due to a MAPT 10+16 mutation in the microtubule associated protein tau, and a second pre-symptomatic case with the same mutation; (ii) [¹⁸F]AV-1451 imaging of a cohort of seven cases with Semantic Dementia and one case of FTD from a C9orf72 expansion, both strongly associated with TDP-43 pathology without tau; and (iii) the increase in [¹⁸F]AV-1451 binding, and changes in the distribution of binding, in thirty one patients spanning the three major FTD syndromes in comparison to matched controls.

The literature on the role of neuroinflammation in FTD is more limited. I used the PET ligand [¹¹C]PK-11195, as an established marker of activated microglia. I report the elevation in [¹¹C]PK-11195 binding, and the change in its distribution, in a case of a pre-symptomatic MAPT 10+16 mutation carrier; and in twenty nine patients spanning the three major FTD syndromes in comparison to matched controls.

In addition to reporting the correlations between PET ligand binding and disease severity, I describe the relationship across regions and across syndromes between [^{18}F]AV-1451 and [^{11}C]PK-11195 binding. In view of the marked variations in affinity of [^{18}F]AV-1451 for different tau isoforms and TDP43-pathology, my analyses focus on multivariate distributions rather than absolute binding potential. The results show high correlations between [^{18}F]AV-1451 and [^{11}C]PK-11195 binding in each FTD syndrome. However, in the healthy MAPT 10+16 carrier, the distribution of elevated [^{11}C]PK-11195 binding is much more extensive than the elevation of [^{18}F]AV-1451, suggesting that inflammation might precede the aggregation of tau.

I discuss the limitations of the PET ligands, and summarise the insights into FTD pathogenesis arising from my series of observational studies. The role of new PET ligands, and the integration of PET in future clinical trials are discussed.

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Abbreviations

A β	β -amyloid
ACE-R	Addenbrooke's cognitive examination - revised edition
AD	Alzheimer's disease
APP	Amyloid precursor protein
ARSAC	Administration of Radioactive Substances Advisory Committee
BP _{ND}	Non-displaceable binding potential
bvFTD	Behavioural variant frontotemporal dementia
C9orf72	C9 open reading frame 72
CANTAB	Cambridge Neuropsychological Test Automated Battery
CBD	Corticobasal degeneration
CBS	Corticobasal syndrome
CHMP2B	Charged multivesicular body protein 2B
CSF	Cerebrospinal fluid
CT	Computer tomography
DeNDRoN	Dementias and Neurodegeneration specialty of the UK Clinical Research Network
FAB	Frontal assessment battery
FTD	Frontotemporal dementia
FTDRS	Frontotemporal dementia rating scale
FTLD	Frontotemporal lobar degeneration
FUS	Fused in sarcoma protein
GBq	Gigabecquerel
GRN	Progranulin
lvPPA	Logopenic variant of primary progressive aphasia
MAPT	Microtubule associated protein tau
MBq	Megabecquerel
MeV	Megaelectron Volt
MMSE	Mini mental state examination
MRI	Magnetic resonance imaging
nfvPPA	Non-fluent variant of primary progressive aphasia
PET	Positron emission tomography
PHF	Paired helical filament
PNFA	Progressive non-fluent aphasia

PPA	Primary progressive aphasia
PSP	Progressive supranuclear palsy
SD	Semantic dementia
SPM	Statistical parametric mapping
svPPA	Semantic variant of primary progressive aphasia
TBK-1	TANK-binding kinase 1
TDP-43	TAR-DNA binding protein 43
TMEM106b	Transmembrane protein 106b
TREM2	Triggering receptor expressed on myeloid cells 2
VCP	Valocin containing peptide

Chapter 1: Introduction

Overview

In this chapter I review the clinical syndromes of Frontotemporal dementia (FTD), along with their neuropathology, genetics and imaging features. I outline the heterogeneity that characterises these disorders, and how this impacts on our understanding of the biology of disease, complicating the development of both symptomatic and disease modifying therapies. Finally, I introduce the concept of using positron emission tomography (PET) as a method of investigating pathophysiology *in vivo* and how this technique may provide valuable insights which may help improve our understanding of the mechanisms of disease and potential avenues for treatment approaches.

Introduction

Frontotemporal dementia (FTD) was described by Arnold Pick in series of cases from 1892, although the earliest clinical description in keeping with frontotemporal dementia may have been that published by William Bevan Lewis from the West Riding Asylum in 1878 (Bevan-Lewis, 1878). The index cases (Pick, 1892, 1901, 1904, 1906) described by Pick illustrated the range of symptoms resulting from what we now call FTD. The pathology associated with FTD was first described in 1911 by Alois Alzheimer who coined the term ‘Pick bodies’ for the characteristic intracellular inclusions. The last hundred years have allowed greater characterisation of the clinical features and neuropathology of these illnesses, in what is now a spectrum of frontotemporal lobar degeneration (FTLD), causing FTD, progressive supranuclear palsy and corticobasal syndrome. FTD is the second commonest cause of young onset dementia after Alzheimer’s disease, and carries a lifetime risk of about 1 in 750 (Coyle-Gilchrist et al., 2016). In up to 40% of cases there is a family history of dementia and in 20% there is a monogenic cause (Bang, Spina, & Miller, 2015; Rohrer, Guerreiro, et al., 2009). Treatment options remain limited, with little evidence base from randomised controlled trials, no licenced therapies for symptom relief and no disease modifying treatments. Furthermore, the heterogeneity of FTD, in both clinical syndromes and in neuropathological subtypes, has posed significant challenges for the understanding of pathophysiology and consequently for the development of treatments. The clinical presentations are diverse and are categorised as either the behavioural variant of FTD (bvFTD) (Rascovsky et al., 2011), or as one of two language variants or primary progressive aphasia (Gorno-Tempini et al., 2011); non-fluent variant primary progressive aphasia (nfvPPA) or semantic variant primary progressive aphasia (svPPA).

In recent years progress has been made in the clinical phenomenology of FTD, in particular refining the diagnostic criteria for the clinical syndromes of FTD and for the underlying neuropathological aetiologies. However, our understanding of the biology of disease and how it relates to the clinical illness remains incomplete, and the dire need for effective therapies to ameliorate the personal, social and economic impact of these diseases unmet. There remain several challenges which confront the FTD research community and prevent us from improving this situation. These challenges take many forms including public and institutional awareness of these relatively rare diseases, a disparity in funding compared with other neurodegenerative diseases such as Alzheimer's disease and motor neurone disease, and the clinicopathological complexity of FTD. In particular this last factor is a prominent barrier to the development of both symptomatic and disease modifying treatments targeting specific aspects of pathophysiology. As well as impacting on drug development and clinical trials, the complex biology of FTD also complicates the identification of pre-symptomatic and diagnostic biomarkers. In order to move clinical management of FTD beyond phenomenology and tertiary specialist clinics and toward the biological diagnosis and treatment of disease, there is in particular a desperate need to develop biomarkers which identify, segregate, quantify, map and track aspects of pathophysiology over time *in vivo*. The substance of this doctoral thesis will be using positron emission tomography (PET) as an approach to address this need.

The clinical syndromes of FTD

Although FTD syndromes have been recognised in various forms for over a century, the interest in the disease and the criteria to define it have varied. In the last 25 years there has been a resurgence of interest in FTD as a neurodegenerative disease causing circumscribed deficits in particular domains whilst sparing others, in other words reflecting the anatomical location of the neurodegenerative damage. In the last 10 years international working parties have established consensus criteria for diagnosis of behavioural and language variants of FTD. In the following sections (summarised in table 1.1) I will present a canonical account of the current clinical and neuropathological classifications as well as genetic aetiologies, highlighting where needed the relevance of older diagnostic approaches particularly when it affects the interpretation of older studies.

<i>Syndrome</i>	<i>Clinical features</i>	<i>Neuropathologies</i>	<i>Genetic aetiologies</i>
BvFTD	3 of: Behavioural disinhibition Apathy or inertia Loss of empathy Stereotyped/ritualistic behaviour Hyperorality Focal executive deficits	TDP-43 (50%) <i>Type A, B, C or D</i> Tau (40%) <i>Pick's, CBD or PSP</i>	C9orf72 expansions GRN mutations MAPT mutations
SvPPA	Impaired confrontational naming Impaired single word comprehension + 3 of: Impaired object knowledge Surface dyslexia Spared repetition Spared speech production	TDP-43 type C (90%) Pick's tauopathy or Alzheimer's pathology (rarely)	Almost never
NfvPPA	1 of: Agrammatic language production Speech apraxia 2 of: Impaired syntactic comprehension Spared single word comprehension Spared object knowledge	Tau (60%) <i>CBD, PSP or Pick's</i> TDP-43 (30%) Alzheimer's pathology (10%)	GRN (rarely)

Table 1.1: summary outline of core clinical features, neuropathology and genetics of each FTD syndrome.

Behavioural variant of FTD

Behavioural variant FTD is characterised by change in personality with behavioural disturbance (Rascovsky et al., 2011). This may involve apathy and withdrawal, disinhibition,

impulsivity, irritability and socially inappropriate behaviour. There are also deficits in theory of mind, social cognition and emotional processing. These features lead to a breakdown in social comportment, interpersonal relationships and self-care. Although not a classical feature of the diagnostic criteria, episodic memory impairment may be seen early on (Hornberger & Piguet, 2012; Rascovsky et al., 2011) and other features such as apraxia or eye movement disorders may emerge later in the disease. Features of anterior horn cell dysfunction occur in up to two fifths of patients, whilst florid motor neuron disease is seen in about 15% (Burrell, Kiernan, Vucic, & Hodges, 2011), with parkinsonism evident in one fifth (Ber et al., 2006).

A genetic cause for this presentation is relatively common with mutations in the three main genes found in FTD known to cause this syndrome: the microtubule associated protein tau (MAPT) gene (Hutton et al., 1998); the Progranulin (GRN) gene (Baker et al., 2006) and the hexanucleotide repeat expansion C9 open reading frame 72 (C9orf72) (Gijssels et al., 2012) accounting for 60% of genetic cases and around 20% of all cases (Rohrer, Guerreiro, et al., 2009).

The pathology in genetic behavioural variant FTD cases is determined by, and typical of, the specific mutation, whilst in sporadic cases pathology it is split almost equally between tau pathology (40%) and TAR-DNA binding protein 43 (TDP-43) pathology (50%). In sporadic cases neuropathology is not accurately predicted by clinical syndrome, although the development of signs consistent with progressive supranuclear palsy increases the likelihood of tau pathology whilst the development of coexistent motor neurone disease or psychosis increases the likelihood of TDP-43 pathology. The course is variable with a mean survival of 4.5 years from diagnosis (Coyle-Gilchrist et al., 2016) and the development of dysphagia and anterior horn cell signs carry a poor prognosis. Treatment is mainly based on anecdotal evidence and directed towards alleviation of carer burden and distress, and amelioration of affective and motor symptoms.

Semantic variant of primary progressive aphasia

The semantic variant of primary progressive aphasia (also called semantic dementia [SD]) (Hodges & Patterson, 2007; Hodges, Patterson, Oxbury, & Funnell, 1992; Hodges et al., 2010; Snowden, Goulding, & Neary, 1989; Warrington, 1975), is characterised by poor semantic knowledge, and whilst a bilateral disease, the syndrome may be predominantly left or right sided in presentation. Left dominant disease is most common, presenting with fluent but empty speech, impaired single word comprehension, anomia, semantic paraphasias and superordinate category errors, as well as surface dyslexia (regularisation of irregular words) on reading. The clinical picture is different in right dominant cases with early prosopagnosia,

impaired emotional recognition and behaviour features such as rigidity and obsessiveness (Chan et al., 2009). Although asymmetric at outset, the syndromes of left and right-semantic dementia become progressively bilateral, affecting behaviour, personality and facial recognition as well as language. Posterior cortical functions are relatively well preserved and motor features are highly unusual (Bang et al., 2015).

There is a strong clinicopathological correlation between the syndrome of semantic dementia and TDP-43 type C pathology (Hodges et al., 2010; Snowden, Neary, & Mann, 2007; Spinelli et al., 2017), which is seen in up to 90% of cases. It is very rare for this type of FTD to have a monogenic basis. The disease has a relatively long course with a mean survival of 9.1 years from diagnosis (Coyle-Gilchrist et al., 2016). Symptomatic pharmacological treatment targets amenable symptoms such as mood and the mainstay of management is social support.

Non-fluent variant of primary progressive aphasia

The non-fluent variant of primary progressive aphasia (Gorno-Tempini et al., 2011), (formerly also called progressive non-fluent aphasia [PNFA]) is characterised by non-fluent, effortful speech which may be agrammatic, with or without apraxia of speech (which rarely may occur in the absence of aphasia (Josephs et al., 2012)) and oro-buccal apraxia on examination. Phonological paraphasias and binary confusion, for example verbally of ‘yes’ and ‘no’, are common features. Reading aloud is often easier than spontaneous speech but is typically aprosodic. Naming is usually normal, whilst other domains such as memory and visuospatial function are well preserved. Features such as eye movement disorders, limb apraxia and extrapyramidal signs may appear during the disease course sometimes to an extent that constitutes corticobasal syndrome or progressive supranuclear palsy (Deramecourt, Lebert, Buee, Maurage, & Pasquier, 2010; Josephs et al., 2006), whilst dysexecutive features and mild behavioural changes may become apparent as the disease progresses.

Non-fluent variant primary progressive aphasia may rarely result from mutations in the GRN gene when the mutation and the disease are associated with TDP-43 type A pathology (Bang et al., 2015; Mackenzie et al., 2011). In sporadic cases the pathology is more commonly tau (Spinelli et al., 2017) but cannot be definitively predicted from the syndrome, although it has been postulated that apraxia of speech may predict the presence of tauopathies such as progressive supranuclear palsy or corticobasal degeneration (Josephs & Duffy, 2008). Mean survival is 8-10 years from symptom onset (Coyle-Gilchrist et al., 2016; Hodges, Davies, Xuereb, Kril, & Halliday, 2003; Spinelli et al., 2017) and whilst the neuropathology underlying the syndrome is heterogeneous this does not seem to impact on prognosis, although there may be differences in behavioural profile and motor features (Spinelli et al.,

2017). Clinical management focuses on managing the frustration of aphasia and preserving other daily functions as far as possible.

Logopenic aphasia

A further subtype of PPA is the logopenic variant of primary progressive aphasia (lvPPA). It is typically early onset in age and presents with language deficits dominated by word finding difficulties and significant anomia which can lead to slow speech with ‘word finding’ pauses. Another marked feature is impaired sentence repetition, thought to result from a deficit in phonological short term memory. Single word repetition and comprehension are typically spared. Later in the disease sentence comprehension may become impaired (Gorno-Tempini et al., 2008). Cognitive decline is faster than other variants of PPA and spreads to involve other domains such as visuospatial function and episodic memory (Leyton, Hsieh, Mioshi, & Hodges, 2012). Whilst the combination of non-fluent speech and speech sound errors may cause confusion with nvPPA, the impairment in confrontational naming and lack of significant agrammatism or speech apraxia usually differentiates the two (Montembeault, Brambati, Gorno-Tempini, & Migliaccio, 2018). Although lvPPA has historically been categorised as a subtype of FTD clinically, it is strongly associated with focal Alzheimer’s pathology (Mesulam et al., 2008; Spinelli et al., 2017), typically present posterior to the perisylvian fissure and into the parietal lobe. Thus it is now generally regarded as a form of focal onset Alzheimer’s disease (Mesulam et al., 2008), in the same way as other focal syndromes of Alzheimer’s disease such as posterior cortical atrophy (Migliaccio et al., 2009). As a result of this strong association with Alzheimer’s disease, lvPPA is not discussed further in this thesis.

For all FTD syndromes, the diagnosis is clinical, based on diagnostic criteria (Gorno-Tempini et al., 2011; Rascovsky et al., 2011) which frame the predominant symptoms and signs at the time of presentation, supported by the pattern of atrophy on MRI (Gordon, Rohrer, & Fox, 2016; Gorno-Tempini et al., 2004; Rohrer et al., 2010), and confirmed by genetic mutation if present (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). Over time symptoms and signs may evolve and broaden so that language variants develop behavioural changes and vice versa, or motor signs become apparent, perhaps reflecting the early localisation of pathology to particular networks with subsequent spread through connected regions (Seeley, 2008). In a similar fashion the labels which best describe an individual’s illness may also change with this pattern of evolution such that the patient presenting with change in personality and behaviour in keeping with behavioural variant FTD who subsequently develops postural

instability and a supranuclear gaze palsy may then be better described as having progressive supranuclear palsy.

Neuropathology in FTD

The morphology and distribution of protein aggregates define the pathological diagnosis and classification of these neurodegenerative diseases. The pathologies which cause FTD fall under the umbrella term of Frontotemporal lobar degeneration. A feature of these pathologies is their heterogeneity, both in the way that more than one pathology can cause the same clinicoanatomical syndrome (e.g. bvFTD may be caused by various subtypes of tau or TDP-43) and in that one specific type of pathology may be the cause of a variety of clinical phenotypes (e.g. Pick's disease pathology may cause bvFTD or nvPPA) (Seeley, 2017).

The protein aggregates which cause FTD syndromes are predominantly of either tau or TDP-43, with neuronal loss affecting the superficial layers of frontal and temporal cortex, with particular severity in layer II. Loss of Von Economo cells occurs in layer Vb and is an early and specific feature of FTD (Seeley, 2008; Seeley et al., 2006). There is also heterogeneity of biochemistry and morphology within the subtypes of protein aggregates observed. Although the predominant proteinopathies are tau or TDP-43, other protein aggregates are occasionally seen. To complicate matters further, there is a poor correlation between clinical phenotype and pathology such that in most cases neuropathology cannot be accurately predicted by clinical syndrome. In the following sections I will briefly describe the characteristics of both tau and TDP-43 aggregation and mention some of the rarer aggregated proteins found in FTD.

Tau

Tau is an intracellular protein primarily involved in the assembly and stabilisation of microtubules and therefore important in axonal transport. It is produced from the MAPT gene found on chromosome 17. Alternative splicing during transcription enables the production of six different isoforms from this single gene, which differ in their microtubule binding domains and which predominate at different stages of brain development. Aggregates of tau are hyperphosphorylated, sometimes with other post-translational modifications, and may be composed predominantly of 3-repeat or 4-repeat isoforms, or a balance of both. Classification of tau pathology is based upon the predominant isoform type, aggregate morphology, cell type affected and distribution of pathology (Dickson, Kouri, Murray, & Josephs, 2011). The three main categories are: *Pick's disease pathology* characterised by intracellular inclusions of 3-repeat tau formed of straight filaments and called Pick bodies; *progressive supranuclear palsy pathology* characterised by predominantly subcortical globose tangles, coiled bodies and tufted astrocytes formed of straight filaments of 4-repeat tau; and lastly *corticobasal*

degeneration pathology, also a 4-repeat tauopathy forming astrocytic plaques, coiled bodies and neuropil threads with a predominantly cortical distribution.

TDP-43

TDP-43 is an intracellular protein which is encoded by the TAR-DNA binding gene found on chromosome 20 (Borroni et al., 2009). It is an important regulator of protein and RNA homeostasis with two main functions: regulation of transcription by its involvement in alternative splicing; and stabilization of RNA molecules. Consequently in health it is localised predominantly to the cell nucleus (Buratti & Baralle, 2008). Aggregates of TDP-43, mislocalised to the cytoplasm, are found in several neurodegenerative diseases but are particularly associated with FTD and motor neurone disease (Warraich, Yang, Nicholson, & Blair, 2010). Most recently, Limbic-predominant age-related TDP-43 encephalopathy (LATE) has been described as another TDP-43 related disease entity typically presenting in older people with an Alzheimer's-like, predominantly amnesic, dementia syndrome, but characterised neuropathologically by the presence of TDP-43 proteinopathy in medial temporal lobe structures (Nelson et al., 2019). The TDP-43 pathology of Frontotemporal lobar degeneration can be divided into four subtypes, *A to D*, based on morphology and distribution (Mackenzie et al., 2011). Recently a type E has been proposed (Lee et al., 2017) representing a more 'virulent' subtype with widespread, immature TDP-43 pathology and a more rapid clinical course.

Less than 10% of cases of FTD examined at *post mortem* have neuropathology which is not tau or TDP-43. The majority of these cases have aggregates of Fused in sarcoma protein (FUS) (Neumann et al., 2009). Aggregates of charged multivesicular body protein 2B (CHMP2B) pathology are the next most recognised finding (Skibinski et al., 2005). Whilst the relationship between pathology and clinical phenotype is weak, there is a far stronger association between the monogenic causes of FTD and neuropathology.

Genetics of FTD

Approximately 40% of people presenting with FTD have a family history of dementia or related neurodegenerative disease and 10% have a strong autosomal dominant family history of FTD (Rohrer, Guerreiro, et al., 2009). Approximately 20% of cases are identifiable as genetic at presentation, with 60% of these genetic cases resulting from a mutation in the MAPT or GRN genes, or from a hexanucleotide repeat expansion in C9orf72 (Bang et al., 2015). With improved understanding of the relationship between genetics and overlapping neurodegenerative phenotypes, it is now recognised that family history of any neurodegenerative disease, especially motor neurone disease, parkinsonism or psychosis, can

be a relevant expression of a mutation in other family members. Apart from C9orf72 and VCP, there are few reliable phenotypic features to indicate genotype or pathology in bvFTD, although brain imaging can be helpful given the association of GRN mutations with extreme asymmetry and of MAPT mutations with symmetrical temporal lobe atrophy respectively (Cash et al., 2018). In the following sections I briefly summarise the characteristics of the main genes, their typical clinical syndromes and pathologies.

Microtubule associated protein tau

The MAPT gene, found on chromosome 17 (Neve, Harris, Kosik, Kurnit, & Donlon, 1986), plays a key role in microtubule homeostasis and therefore in axonal transport. Tau is a microtubule associated protein which undergoes a wide range of post translational modifications controlling its behaviour. Its primary function in normal neuronal physiology is the stabilization of microtubules through which it has effects on key neuronal processes such as axonal transport. More recently a role for tau has been postulated in long term synaptic plasticity, specifically in the activity dependent decrease in synaptic activity known as long term depression (Medina, Hernández, & Avila, 2016). The MAPT gene contains 16 exons. Alternative splicing of exons 2, 3 and 10 results in the expression of 6 different tau isoforms (Goedert, Spillantini, Jakes, Rutherford, & Crowther, 1989). Inclusion or exclusion of exon 10 produces a protein with 4 or 3 repeat binding domains and normal tau function requires a balance of 3 and 4 repeat isoforms. Whilst integral to normal neuronal function, aggregates of tau are characteristic of several neurodegenerative diseases. Mutations in MAPT were the first described genetic cause of FTD (Hutton et al., 1998) and *post mortem* examination reveals subtypes of tau pathology which vary by mutation and encompasses the biochemical and morphological spectrum of tau aggregation (Cairns, Lee, & Trojanowski, 2004). The clinical presentation is generally behavioural, may occur with or without parkinsonism, and has been described in association with other presentations of frontotemporal lobar degeneration such as progressive supranuclear palsy (Morris et al., 2003). Although rarer, semantic (Bessi et al., 2010), non-fluent (Munoz, Ros, Fatas, Bermejo, & Yebenes, 2007) and amnesic presentations are described.

C9orf72

C9orf72 is found on chromosome 9. The role of the C9orf72 protein is not fully understood but it is found pre-synaptically and seems to play an import role in endosomal trafficking, particularly in neurons (Farg et al., 2014). A hexanucleotide repeat length greater than thirty appears to lead to both loss of function, primarily affecting immune cells (Lall & Baloh, 2017; O'Rourke et al., 2016), and a toxic gain of function related to the generation of sense

and anti-sense RNA species, and of toxic dipeptide repeat proteins (Moens, Partridge, & Isaacs, 2017). These repeat expansions are unstable and vary between tissues but are not fully penetrant and while the effect of expansion size on onset age and disease severity remains unclear, it appears that repeat length is not predictive (Benussi et al., 2014; Blitterswijk et al., 2013).

The neuropathology is classically TDP-43 aggregates, predominantly of type B (Mackenzie et al., 2011), although very rare cases with additional tau pathology have been described (Snowden et al., 2012). The pathological hallmark of C9orf72 disease is the presence of dipeptide repeat aggregates, formed by unconventional repeat-associated non-ATG translation (Ash et al., 2013; Mori et al., 2013; Rohrer et al., 2015). It is the most recently described gene to account for a large number of cases of FTD (Dejesus-Hernandez et al., 2012; Renton et al., 2011), for example a recent European study found C9orf72 expansions to account for 10% of all frontotemporal lobar degeneration cases, almost 20% of familial cases and 6% of sporadic cases (van der Zee et al., 2012).

The cognitive phenotype is most often behavioural, however expansions have also been associated with amnesic, non-fluent and semantic presentations. Psychotic features are more common than in sporadic behavioural variant FTD (Devenney et al., 2014) and psychiatric symptoms may predominate early in an illness such that psychiatric diagnoses like obsessive compulsive disorder, schizophrenia, bipolar disorder and depression often precede the diagnosis of FTD (Cooper-Knock, Shaw, & Kirby, 2014; Lindquist et al., 2013). Motor features are common, with parkinsonism present in up to one third of cases (Rohrer & Warren, 2011). Although occurring sporadically and with other mutations, the FTD-motor neurone disease complex is strongly associated with expansions in C9orf72 (Burrell et al., 2016; Snowden et al., 2012). The phenotype can be pure motor neurone disease, of which expansions in C9orf72 are the most common genetic cause (Farg et al., 2014). Expansions in C9orf72 have also been found in people with clinical syndromes consistent with Alzheimer's disease, corticobasal syndrome, parkinsonian illnesses including dementia with Lewy bodies, Huntington's disease and progressive ataxias (Cooper-Knock et al., 2014; Lindquist et al., 2013). Consequently phenotypic variability may be seen even within the same family.

Progranulin

GRN is found on chromosome 17 and consists of 13 exons which produce a precursor protein that has diverse immune regulatory functions. These include potential antagonism of the tissue necrosis factor receptor and autocrine functions, as well as playing a role in lysosome homeostasis (Tang et al., 2011; Toh, Chitramuthu, Bennett, & Bateman, 2011). It therefore

has a broad range of effects on both neurons and glial cells (Ahmed, Mackenzie, Hutton, & Dickson, 2007; Filiano et al., 2013). Mutations in GRN are associated with haploinsufficiency and reduced concentrations in both serum and CSF (Gijselinck, Van Broeckhoven, & Cruts, 2008; Shankaran et al., 2008). The neuropathology of GRN mutations is characterised by TDP-43 aggregation of type A (Mackenzie et al., 2011). It was first described as a cause of FTD in 2006 (Baker et al., 2006; Cruts et al., 2006) and most commonly presents as a predominantly behavioural syndrome or with a non-fluent aphasia (Chen-Plotkin et al., 2011; Yu et al., 2010). Features of motor neurone disease are rare but parkinsonism can occur (Rohrer & Warren, 2011). The phenotype may show variability even within the same family (Gabryelewicz et al., 2010).

Other monogenic causes

Other genetic causes are rare and the remainder of cases result from less common mutations in genes such as CHMP2B, FUS, TBK-1 and VCP, although these make up less than 5% of genetic cases. In addition, mutations in presenilin 1, presenilin 2, Prion, TDP-43 and others can rarely present with FTD-like phenotypes (Blauwendraat et al., 2018). Given the high rates of family history of FTD, dementia, or other neurodegenerative disease in individuals with FTD it seems likely there are further monogenic causes yet to be discovered, particularly in phenotypes such as FTD – motor neurone disease (Burrell et al., 2016).

Other genetic factors

The importance of genetics is not only clinical but also provides insights into the mechanisms of disease and consequently into potential therapeutic approaches. The role of genes beyond monogenic aetiology is therefore important. Alongside proposed cognitive factors which may impact on the course of dementia (Stern, 2009), other genetic factors seem likely to account for the differences in onset, progression and phenotype seen even within families with the same FTD-causing mutation. Polymorphisms in several different genes have been recognised to confer risk or protection in frontotemporal lobar degeneration. For example, progressive supranuclear palsy and corticobasal degeneration, the pathologies of which may account for behavioural and non-fluent presentations of FTD, are more common in individuals with the H1 haplotype of the MAPT gene, a finding reproduced in a clinical cohort with FTD (Baker et al., 2018; Verpillat et al., 2002).

Another important gene which modulates risk is Transmembrane protein 106b (TMEM106b), a protein involved in lysosomal function, single nucleotide polymorphisms of which have been identified from recent genome-wide association studies as risk alleles in FTLTDP43 (Van Deerlin et al., 2010), particularly in cases due to mutations in C9orf72 or GRN

(Cruchaga et al., 2011; Gallagher et al., 2014; Van Blitterswijk et al., 2014; Van Deerlin et al., 2010). The risk allele of TMEM106b has also been shown to modulate the effect of environmental factors such as education on grey matter volume in carriers of C9orf72 hexanucleotide repeat expansions (Premi et al., 2017), and to correlate with structural changes in healthy carriers (Adams et al., 2014) as well as with grey matter volume in homozygous carriers with FTD (Harding et al., 2017).

Genes related to immune function have also been implicated as risk factors for FTD by genome-wide association studies (Ramanan & Saykin, 2013). Amongst these is a variant of triggering receptor expressed on myeloid cells 2 (TREM2), a protein which is important in regulation of phagocytosis by activated microglia and which confers significant risk for Alzheimer's disease, whilst a variant in Interleukin 1- β is associated with an earlier age of onset of bvFTD. Apolipoprotein E alleles have also been associated with FTD and have also previously been shown to modulate neuroinflammation through effects on inflammatory receptor signalling to a pro-inflammatory phenotype (Tai et al., 2015). Building on the potential mechanistic importance of inflammation in FTD, in the next section I will discuss neuroinflammation more generally before focusing on the evidence for neuroinflammation in FTD.

Neuroinflammation

The presence of innate immune functions in the central nervous system, and particularly the concept of neuroinflammation, is increasingly recognised as a potentially important and modifiable component in the process of neurodegeneration. However, despite many studies of population genetics, *post mortem* tissue and animal models, the understanding of the presence and timing of *in vivo* neuroinflammation in neurodegenerative disease remains incomplete.

Until recently the brain was often regarded as an 'immune privileged' organ, that is one where inflammation can only occur in the context of direct infection or after breakdown of the blood brain barrier allowing migration of peripheral immune cells. However, it is now known that cell types within the brain express pattern recognition receptors which can activate inflammatory signalling pathways in response to particular pathogen associated molecular patterns, thus providing a potential basis for intrinsic neuroinflammation. Linking this intrinsic neuroinflammation with neurodegeneration is evidence that misfolded proteins may be able to trigger these pathways in a similar manner by acting as danger associated molecular patterns, in essence causing 'sterile' neuroinflammation (Graeber, Li, & Rodriguez, 2011; Heneka, Kummer, & Latz, 2014). Within this world of central nervous system inflammation,

microglia play a key role (Nakajima & Kohsaka, 2001). Therefore, when referring to neuroinflammation in this thesis I will be implying the presence of activated microglia unrelated to an extrinsic stimulus. Microglia are the resident macrophages of the central nervous system and are sensitive to brain injury and disease becoming activated by a wide range of pathophysiological insults (Nayak, Roth, & McGavern, 2014). They are found in differing densities throughout the brain and their phenotype differs depending on localization. Through their surveillant, pruning, phagocytic, proliferating and neuromodulatory phenotypes, microglia can effect neurons, astrocytes and blood vessels and play a major role in normal brain homeostasis, influencing cerebral tissue maintenance and neuronal plasticity (Gomez-Nicola & Perry, 2015). These effects are important in neurodevelopment, adult brain homeostasis, and are likely to be important in neurodegeneration. In the healthy adult brain the microglial phenotype is predominantly surveillant and highly sensitive to almost any disturbance of central nervous system homeostasis, being activated through Toll-like receptor signalling pathways (Carpentier, Duncan, & Miller, 2008) by a range of pathophysiological insults (Nakajima & Kohsaka, 2001; Nayak et al., 2014). These activated microglia may then alter brain homeostasis through several mechanisms including inflammatory pathways, effects on plasticity and cytotoxic effects, the purpose of which are to eradicate the stimulus and repair damage (Banati, 2002a; Banati, Gehrmann, Schubert, & Kreutzberg, 1991). In the context of neurodegenerative disease, where aggregated protein species may be the triggering danger associated molecular patterns, these effects may modulate the subsequent course of the neurodegenerative cascade in either a protective or, as this state of activation may become chronic, ineffective and potentially toxic, a deleterious manner (Gomez-Nicola, Fransen, Suzzi, & Perry, 2013; Pasqualetti, Brooks, & Edison, 2015; Serrano-Pozo, Betensky, Frosch, & Hyman, 2016).

Neuroinflammation in neurodegenerative disease

Evidence for neuroinflammation in FTD comes from *post mortem* studies of pathology in patients, studies of pathology in animal models, genome-wide association studies, epidemiological studies and from studies of biomarkers such as cerebrospinal fluid and PET. However, the vast majority of research into neuroinflammation in neurodegenerative disease has been outside of FTLD in illnesses such as Alzheimer's disease, Parkinson's disease and motor neuron disease. Whilst the exact mechanisms of neuroinflammation may not be the same across different neurodegenerative diseases, there are many parallels which may be drawn (Guillot-Sestier & Town, 2018). It has been shown that aggregated protein species can activate microglia (Heneka et al., 2014), and that a robust innate immune response characterised by activated microglia is part of the neuropathology of several

neurodegenerative diseases (Lant et al., 2014; Venneti, Wang, Nguyen, & Wiley, 2008). There is also evidence of a pro-inflammatory profile of cytokines in the brains of people with neurodegenerative disease (Heneka et al., 2015). The hypothesis of a role for neuroinflammation in neurodegenerative disease was supported by epidemiological studies of Alzheimer's disease which found a risk reduction in those using long term anti-inflammatory medication (McGeer & McGeer, 2007; McGeer & McGeer, 2013). Genome-wide association studies have also implicated genes with immune function in several neurodegenerative illnesses including Alzheimer's disease, Parkinson's disease and motor neuron disease (Ramanan & Saykin, 2013). Animal models of these diseases have demonstrated that inflammatory changes precede and interact with other features of neurodegeneration (Krabbe et al., 2013; Streit, Braak, Xue, & Bechmann, 2009), form a major component in the evolution of chronic neurodegeneration and if modulated can affect the disease course (Bhaskar et al., 2010; Martínez-Muriana et al., 2016; Nash et al., 2013; Spangenberg et al., 2018). They have also shown the potential interplay between systemic inflammation and the innate immune response in the brain (Cunningham et al., 2009).

Despite this cumulative evidence, the exact inflammatory phenotype of individual neurodegenerative diseases remains elusive and there have been conflicting reports of whether a pro- or an anti-inflammatory phenotype predominates in Alzheimer's disease (Brosseron, Krauthausen, Kummer, & Heneka, 2014).

Neuroinflammation in FTD

There is also converging evidence to support activation of innate immunity in the central nervous system in FTD. First, a robust innate immune response characterised by activated microglia is part of the neuropathology of FTD and the pattern of this neuroinflammation, as with protein aggregation, differs in FTD from Alzheimer's disease (Lant et al., 2014).

Genetic studies have also indicated a link between the immune system and FTD, in particular the finding that mutations in the GRN gene, whose product is a protein with diverse immune functions, lead to TDP-43 neuropathology and FTD (Ahmed et al., 2007). Whilst the exact pathological cascade is uncertain it seems likely that haploinsufficiency of progranulin results in dysregulated microglial activation and TDP-43 accumulation (Kleinberger, Capell, Haass, & Broeckhoven, 2013). More recently mutations in TBK-1, a protein involved in the regulation of interferon anti-viral responses, has also been demonstrated as a genetic cause of FTD (Gijssels et al., 2015; Oakes, Davies, & Collins, 2017). Genome-wide association studies have also provided more indirect evidence for immune dysfunction in FTD with several immune loci identified in large studies as risk factors for FTD (Broce et al., 2018).

Many of these are the same sites identified for other neurodegenerative diseases (Ramanan & Saykin, 2013).

Mouse models of FTD also support an early role for neuroinflammation. Mouse models of FTD tauopathy feature inflammatory changes which precede the accumulation of aggregated tau (Yoshiyama et al., 2007), and a pro-inflammatory profile of molecules which increase tau hyperphosphorylation and aggregation (Bhaskar et al., 2010). Fractalkine, an endogenous chemokine which modulates microglial activity, reduces this microglial activation and subsequent tau aggregation (Nash et al., 2013). Mouse models of CHMP2B FTD also show microglial activation and a marked pro-inflammatory state preceding neuronal loss and behavioural symptoms, with a similar chemical inflammatory profile seen in human brain *post mortem* in CHMP2B disease (Clayton et al., 2017, 2015). Mouse models of C9orf72 disease, the most common genetic cause of FTD, show that C9orf72 appears to be required for proper microglial function (O'Rourke et al., 2016).

Studies in humans add further weight to the role of neuroinflammation. Epidemiology reveals an increased incidence of autoimmune disease in individuals with FTD due to TDP-43 aggregates and also in C9orf72 expansion carriers (Miller et al., 2013, 2016). Studies of cerebrospinal fluid in FTD have demonstrated increased levels of tissue necrosis factor and transforming growth factor β (Sjogren, Folkesson, Blennow, & Tarkowski, 2004), the latter has also been found to be increased in the plasma of individuals with semantic variant PPA and FTD due to GRN mutation (Kleinberger et al., 2013). Whilst PET studies using markers of activated microglia are few and on a small scale, previous efforts have suggested increased levels of activated microglia in the frontotemporal regions of participants with FTD compared with healthy volunteers (Cagnin, Rossor, Sampson, MacKinnon, & Banati, 2004), and have shown microglial activation in asymptomatic carriers of MAPT mutations (Miyoshi et al., 2010).

Imaging and biomarkers with relevance to FTD

Until the advent of non-invasive brain imaging (CT, then MRI) researchers relied largely on *post mortem* studies to determine the topography of neurodegeneration. The human brain mapping made possible by MRI has enabled identification of prototypical patterns of disease in Alzheimer's disease and FTD. In many ways, our current understanding of the molecular pathophysiology of FTD is similar to that of the topography of atrophy in FTD prior to the advent of MRI. Although there are strong hypotheses about protein aggregation and neuroinflammation, based primarily on pathological findings, our knowledge of mechanisms

of toxicity and relationships to clinical symptoms are drawn largely from *in vitro* and animal models (Ling, Polymenidou, & Cleveland, 2013; Tracy & Gan, 2018).

The development of imaging techniques as mechanistic biomarkers would enable stratification and quantification of pathology *in vivo*, in the same way that atrophy and functional measures have been investigated, and would be a huge step forward as it would allow fundamental questions regarding this component of pathophysiology and its relationship to downstream events to be addressed. In the following sections I will summarise the structural findings from studies in FTD and introduce the role for PET studies to approach important questions regarding molecular processes in FTD.

Structural magnetic resonance imaging findings in FTD

Both structural and functional imaging methods have been used to study FTD. Here I will focus on the structural studies which have correlated atrophy patterns with clinical syndrome, genetic diagnosis and pathological subtype. Rates of atrophy have also been calculated (Gordon et al., 2010; Knopman et al., 2009; Rohrer et al., 2008; Rohrer, Clarkson, Kittus, Rossor, & Ourselin, 2012; Whitwell et al., 2015) and may be useful in assessing response to future therapies. These imaging abnormalities, of brain structure or function, have also become part of the diagnostic criteria for behavioural variant FTD as they improve specificity (Rascovsky et al., 2011).

Behavioural variant FTD

At a group level, imaging in these patients reveals predominantly frontal atrophy affecting the prefrontal cortex, anterior cingulate cortices, insula, anterior temporal lobes, striatum and thalamus (Gordon et al., 2016; Pan et al., 2012; Schroeter, Raczka, Neumann, & Cramon, 2007). The earliest atrophy may be seen in paralimbic cortices before spreading to through the paralimbic fronto-insular-striatal network (Rabinovici et al., 2008; Seeley et al., 2008). A typical atrophy pattern for an individual with bvFTD is shown in figure 1.1. The pattern of atrophy correlates more closely with clinical features (Hornberger, Geng, & Hodges, 2011; Hornberger et al., 2014; Lagarde et al., 2013; Woolley et al., 2007) than with underlying neuropathology (Piguet, Hornberger, Mioshi, & Hodges, 2011), in keeping with the fact the clinical features are a poor predictor of neuropathology across the FTD spectrum, and confirming the clinicoanatomical relationship, i.e. that symptoms reflect the location of damage. Whilst these findings are consistent at a group level, it is striking how much heterogeneity there is between individual patients, with variable symmetry and frontotemporal lobar predominance (Gordon et al., 2016). It has been postulated that this heterogeneity is

underpinned by four main neuroanatomical subtypes of behavioural variant FTD (Whitwell et al., 2009) but it is also likely to reflect the heterogeneity in clinical phenotype between individuals with bvFTD.

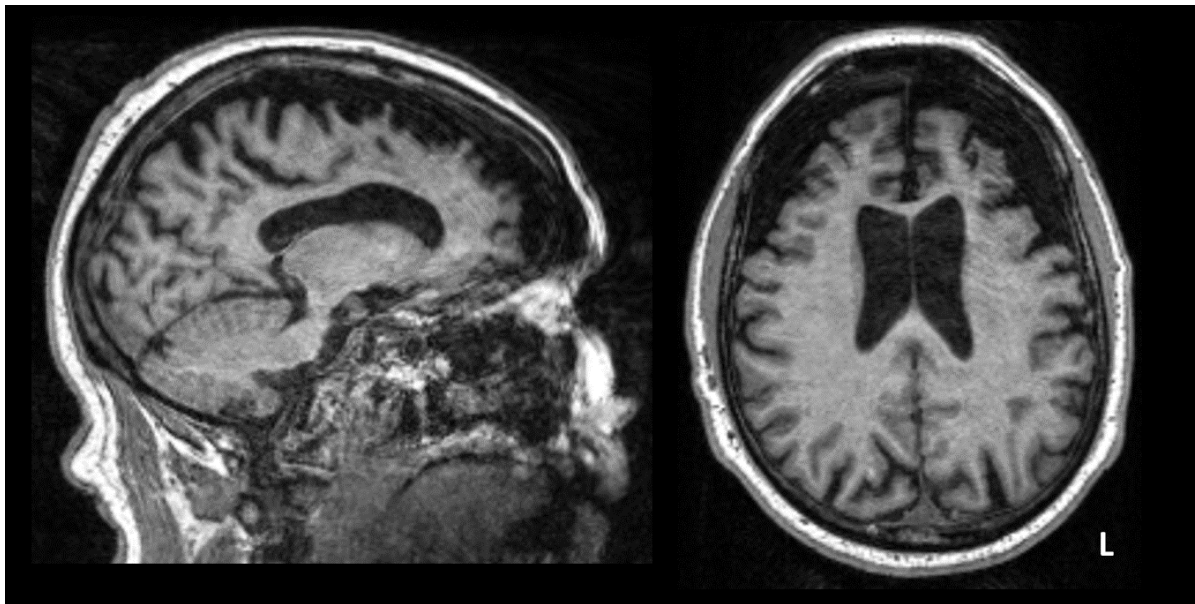


Figure 1.1: Sagittal and axial views demonstrating frontal atrophy in a typical bvFTD participant.

Semantic variant of primary progressive aphasia/ Semantic Dementia

As with clinical phenotype, imaging findings are the most consistent at single subject level in semantic dementia. Typically there is atrophy of the temporal lobes which is bilateral but asymmetrical, particularly affecting the anterior, inferior temporal lobe (Gorno-Tempini et al., 2004; Mummery et al., 2000; Rohrer, Warren, et al., 2009). The typical pattern of atrophy for an individual with svPPA is shown in figure 1.2. The earliest changes include atrophy in the temporal pole, inferior temporal lobe, parahippocampus and fusiform gyri (Brambati et al., 2009; Rogalski, Cobia, Harrison, Wieneke, Weintraub, et al., 2011). Structures such as the amygdala (Chan et al., 2009; Schroeter et al., 2007), insula, entorhinal cortex (Brambati et al., 2009; Rosen et al., 2002) and hippocampus (Chan et al., 2001; Chan et al., 2009; Galton et al., 2001) may also be involved. Left predominance is the more common finding with atrophy progressing to eventually become more symmetrical (Kumfor et al., 2016) and extending to involve frontal and cingulate as well as temporoparietal cortices (Rohrer, Warren, et al., 2009). Even in left dominant cases the same pattern of atrophy is seen in the right temporal lobe (Chan et al., 2001). The predominance of atrophy mirrors the clinical syndrome (Chan et al., 2009) and clinical measures of verbal and visual recognition correlate with left temporal lobe and right temporal lobe atrophy respectively (Chan et al., 2001).

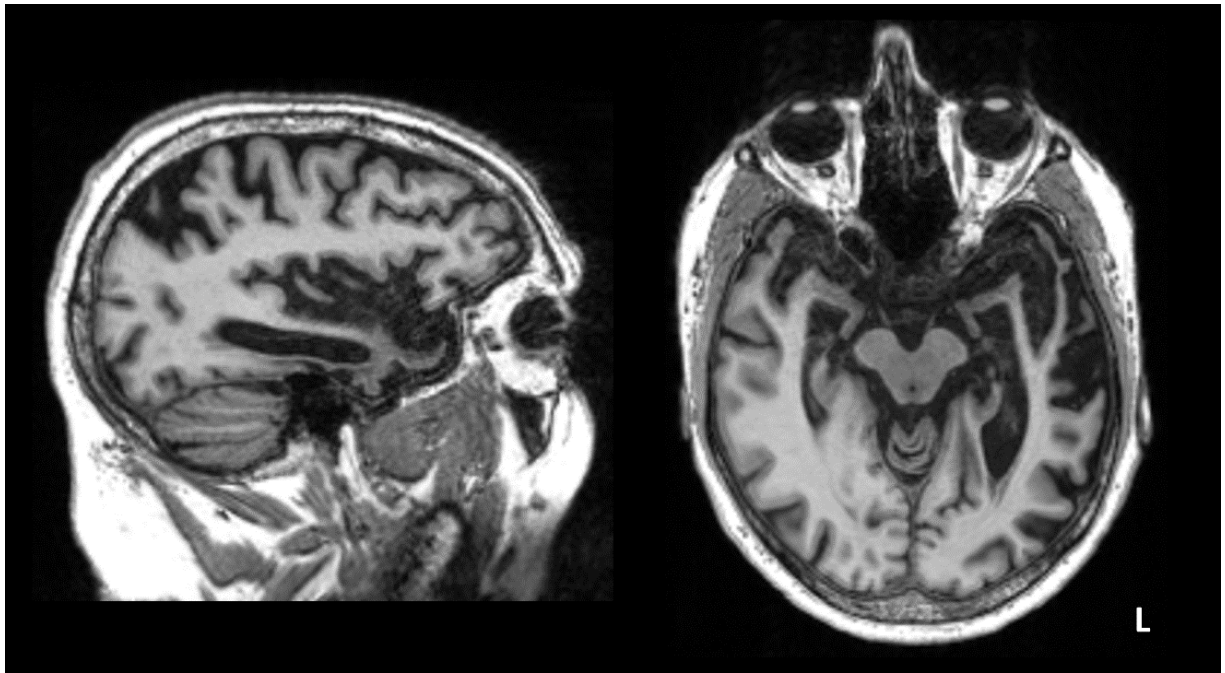


Figure 1.2: Sagittal and axial views demonstrating the severe anterior inferior temporal atrophy typical in semantic dementia.

Non-fluent variant of primary progressive aphasia

Clinical imaging in these patients is often normal, as illustrated in figure 1.3, but voxel based morphometry studies have shown cortical loss in the left hemisphere with a pattern which is distinct from semantic variant primary progressive aphasia (Gorno-Tempini et al., 2004; Rogalski, Cobia, Harrison, Wieneke, Weintraub, et al., 2011). Changes include perisylvian atrophy (Nestor et al., 2003), and volume loss in dorsolateral prefrontal cortex, inferior frontal gyrus (Gorno-Tempini et al., 2004; Josephs et al., 2006), superior temporal gyrus, anterior parietal lobe and insula, as well as in prefrontal areas, and subcortical nuclei such as the caudate and putamen. Changes are also seen in right hemisphere structures with advancing clinical severity (Gordon et al., 2016; Seelaar, Rohrer, Pijnenburg, Fox, & van Swieten, 2011). It is hypothesised that apraxia of speech is particularly associated with atrophy in pre-motor and supplementary motor areas whilst non-fluent aphasia is associated with perisylvian atrophy (Josephs et al., 2006; Josephs et al., 2012; Josephs & Duffy, 2008).

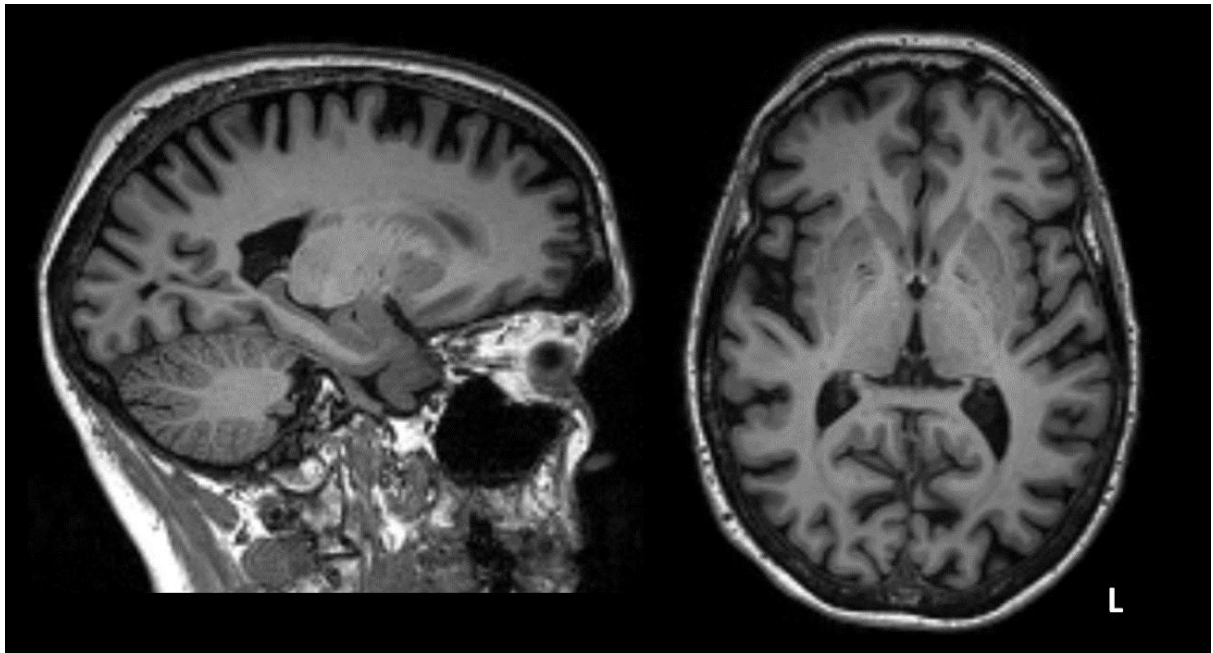


Figure 1.3: Axial and sagittal images demonstrating the relatively normal single subject images in a typical participant with *nvPPA*

Structural imaging in familial FTD

Recent large scale studies of genetic FTD have taken similar approaches in individual monogenic aetiologies and even within specific mutations. Two main questions are of particular importance in this setting. First, do separate genetic variants, both different genes and pathogenic variants within a specific gene, have specific patterns of atrophy? Second, when does this atrophy appear and can it be used to as a biomarker?

Addressing the first question, it is becoming apparent that particular genetic aetiologies have a pattern of atrophy at a group level typical to the mutation. For example in *MAPT* mutations, anteromedial temporal lobe atrophy is typically symmetrical (Josephs et al., 2009; Rohrer et al., 2010; Whitwell et al., 2012), while *GRN* mutations lead to very asymmetrical temporoparietal atrophy (Josephs et al., 2009; Rohrer et al., 2010; Whitwell et al., 2012). *C9orf72* hexanucleotide expansions are associated with widespread symmetrical atrophy, most marked in dorsolateral and medial frontal lobe and orbitofrontal cortex, but also seen in anterior temporal lobes, parietal lobe, occipital lobe, thalamus and cerebellum (Whitwell et al., 2012). Whilst each mutation group exhibits unique regions of atrophy which define these typical patterns, across all groups the atrophy affects a common network of regions including insula, orbitofrontal cortex and anterior cingulate cortex (Cash et al., 2018). It is also clear that the role of cerebellar atrophy is particularly relevant in monogenic forms of FTD where

differential patterns of cerebellar atrophy are also associated with different monogenic causes (Bocchetta et al., 2016; Rohrer et al., 2015; Whitwell et al., 2015; Whitwell et al., 2012).

The second question has potentially significant implications for diagnosis, and both as a selection and outcome measure for clinical trials of treatments in genetic subtypes of FTD. It is a question which is not easily answered due to the heterogeneity of mutations in the same gene and the relatively small effects seen in the years prior to symptom onset. However, there is now evidence that structural changes are found in pre-symptomatic mutation carriers affecting both white matter and grey matter. These are present in all genetic groups, becoming apparent up to 10-15 years before symptom onset and progressing towards conversion to symptomatic FTD (Cash et al., 2018; Jiskoot, Bocchetta, et al., 2018; Jiskoot, Panman, et al., 2018; Rohrer et al., 2015).

Structural imaging in pathological subtypes

There has been great interest in trying to determine the imaging patterns of specific pathological subtypes of FTLT given the potentially important role of protein specific therapies as disease modifying treatments in the near future. Within the *FTLT-tau* group, *Pick's disease* pathology has been associated with asymmetric atrophy of the frontal cortex, anterior temporal lobes and insula (Rankin et al., 2011; Whitwell et al., 2011). The four-repeat tauopathies of *progressive supranuclear palsy* pathology and *corticobasal degeneration* are associated with greater atrophy in the supplementary and pre-motor areas, with a more distributed pattern seen in *corticobasal degeneration* involving the parietal lobes (Josephs et al., 2008; Whitwell, Jack Jr, Boeve, et al., 2010). In the *FTLT-TDP* group, *TDP-43 type A*, which is commonly associated with GRN mutations, has been correlated with a similarly asymmetric pattern of frontotemporoparietal atrophy (Rohrer et al., 2010; Whitwell, Jack Jr, Parisi, et al., 2010). In contrast *TDP-43 type B* pathology is associated with symmetrical frontal lobe atrophy which also affects the anteromedial temporal lobes and insula, whilst *TDP-43 type C* is associated with asymmetrical anteroinferior atrophy in the temporal lobes (Rohrer et al., 2011), itself strongly associated with the clinical diagnosis of semantic dementia. *TDP-43 type D* is rare and commonly found in disease caused by VCP mutations, studies to date have failed to reveal a consistent pattern of atrophy (Gordon et al., 2016). Whilst FUS pathology is also rare, it features striking caudate atrophy and has been associated with volume loss in orbitofrontal and anterior cingulate cortices, anteromedial temporal lobe and insula (Josephs et al., 2010; Seelaar, Klijnsma, Koning, Lugt, & Swieten, 2010). Despite these findings, in essence the pattern of atrophy reflects the clinical syndrome better than the underlying pathology.

PET studies for molecular imaging

Whilst determining patterns of atrophy in neurodegenerative in vivo has improved our understanding of these diseases, structural imaging illustrates the downstream effects of disease and does not provide us the necessary insights into the biology and mechanisms of neurodegenerative processes. In order to improve our understanding of these aspects of disease during life we need imaging techniques which allow us to visualise molecular biological processes. One way of doing this is by using PET, an imaging modality which exploits bespoke molecules labelled with a radioactive isotopes, such as Carbon-11 and Fluorine-18, to target specific processes of interest, termed radioligands. These radioligands have an unstable nucleus with an excess of protons and as these protons decay they emit a positron and a neutrino. The emitted positrons interact with the surrounding electrons resulting in annihilation events which converts the mass energy of the positron and electron to electromagnetic energy in the form of two anti-parallel photons travelling in almost opposite directions. These photons which result from annihilation events and be detected using a PET camera with scintillation detectors in coincidence mode, such that when two opposing detectors detect the annihilation photons within a very small time frame (in the order of 15 nanoseconds) they are assumed to originate from the same annihilation event, the site of which can be determined to be along a line of projection connecting the detectors. The spatial resolution of the detectors and the kinetic energy of the positrons determine that the spatial resolution of PET in humans is usually several millimetres. This data can be acquired across time frames which can each be independently reconstructed and then corrected in a kinetic model. This is particularly useful as radioligands are distributed through human tissues over time and allows accurate quantification and localisation of a signal from specific molecular targets.

PET is now well established as a research tool and in some areas in clinical medicine. It has been used in neurodegenerative research for many years, including previously in FTD. However, the majority of PET studies thus far have targeted downstream effects of neurodegeneration such as changes in metabolism or in neurotransmitters. Given their predicted specificity for molecular targets of interest, PET ligands have potential utility as biomarkers of upstream events, such as protein aggregation and microglial activation. There is a desperate need in FTD to follow the lead of β -amyloid imaging in Alzheimer's disease, particularly as the clinical and pathological heterogeneity of FTD mean that drug discovery and clinical trials are challenging, to an even greater extent than in Alzheimer's disease. Validation of robust PET ligands for use as biomarkers would accelerate and de-risk clinical trials, providing tools for stratification and surrogate outcome measures. Ideal biomarkers of

pathology for disease modifying drugs trials would be selective, that is sensitive to and specific for, the relevant components of pathophysiology. They would ideally correlate with the disease phenotype and clinical severity and would be able to track the quantity and distribution of pathophysiology with disease progression. As the importance of tau and neuroinflammation in neurodegeneration has emerged, there has been great interest in developing specific compounds which could enable visualisation of these processes *in vivo*. In the following section I will give an over view of the background to radioligands in this setting.

In vivo study of neuroinflammation using PET

The potential mechanistic contribution of neuroinflammation to neurodegeneration has prompted the development of several PET ligands to image activated microglia *in vivo*. All of these utilise binding to the translocator protein (TSPO – also known as the peripheral benzodiazepine receptor), which is upregulated on the mitochondrial membrane of microglia in the activated state and thus is a marker of activated microglia. The first radioligand developed to bind this target was [¹¹C]PK-11195. This ligand has been widely used in studies of the central nervous system, in particular in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and dementia with Lewy bodies, where it has provided evidence for *in vivo* neuroinflammation in neurodegeneration (Cagnin, Kassiou, Meikle, & Banat, 2006; Edison et al., 2013; Koh, 2008; Passamonti et al., n.d.; Varley, Brooks, & Edison, 2014). It is highly robust as it is selective for activated microglia over quiescent microglia and reactive astrocytes (Banati, 2002b), is less affected by common polymorphisms in its target protein than its second generation relatives (Stefaniak & Brien, 2015; Zhang, 2015), and has robust methods of non-invasive kinetic analysis (Passamonti et al., n.d.; Turkheimer et al., 2007). Whilst there are some suboptimal features to [¹¹C]PK-11195, the other more recent radioligands which target TSPO also suffer similar problems and lack options for non-invasive modelling of the PET data. Therefore, [¹¹C]PK-11195 remains the most robust and widely used radioligand to image neuroinflammation in neurodegenerative disease.

PET imaging of tau pathology

The importance of tau in neurodegeneration has led to concerted efforts to develop radioligands enabling visualisation of its quantity and distribution *in vivo*. Like all good radioligands, candidate compounds must cross the blood-brain barrier, have low toxicity and low non-specific binding, as well rapid uptake by, and clearance from, the brain without retaining radiolabelled metabolites. There are however several additional challenges to imaging tau (Laforce Jr et al., 2018; Lois, Gonzalez, Johnson, & Price, 2018; Villemagne,

Fodero-Tavoletti, Masters, & Rowe, 2015; Villemagne & Okamura, 2016). These include the need for the radioligand to cross the cell membrane to reach intracellular tau pathology and the fact that tau pathology is found at lower concentrations in the brain than β -amyloid. The heterogeneity of tau pathology is also a significant factor along with other post translational modifications and the maturity of tau tangles which may both also be relevant (Lowe et al., 2016).

Several ligands have been developed to bind to paired helical filament tau in Alzheimer's disease. These include families of compounds such as THK and PBB (Laforce Jr et al., 2018) but the most widely studied tau binding radioligand to date is the benzimidazole-pyrimidine derivative, [^{18}F]AV-1451. This compound is selective for tau *versus* β -amyloid and *versus* α -synuclein (Xia et al., 2013). It has strongly convincing results *in vivo* in Alzheimer's disease, where binding recapitulates Braak staging (Schwarz et al., 2016) and correlates strongly with phenotypic variation (Ossenkoppele et al., 2018, 2016; Scholl et al., 2017), as well as with clinical severity. More recently, longitudinal studies have shown measurable increases in amount of binding over time as well as changing distribution, most obvious in cognitively impaired and β -amyloid positive individuals (Jack et al., 2018; Lowe et al., 2018). This tracer also has some drawbacks including age-related increases in binding (Johnson et al., 2016; Lowe et al., 2018; Scholl et al., 2016) as well as potential 'off target' binding to neuromelanin, and to iron and other mineral targets (Lowe et al., 2016; Marquie et al., 2015, 2017). Despite these issues, it remains the most robust 'tau ligand' available in Alzheimer's disease. At the onset of this study relatively little was known about its ability to bind to tau pathology in FTD but it was the foremost candidate to be investigated for sensitivity and specificity in this non-Alzheimer's setting.

Conclusion

In this chapter I have summarised the clinical, pathological, genetic and imaging features highlighting the broad and complex spectrum of FTD. I have also introduced some of the challenges facing the FTD community and how the use of molecular PET may help to unravel some of this complexity and address major challenges in improving our understanding of FTD.

This thesis focuses on two important and likely complimentary pathophysiological processes, tau aggregation and neuroinflammation. To do this I use two PET tracers, [^{11}C]PK-11195 (a marker of microglial activation and a proxy of neuroinflammation) and [^{18}F]AV-1451 (a marker of tau pathology).

The objectives of this are twofold;

- (1) First to investigate whether [^{18}F]AV-1451 PET in FTD enables the segregation of FTD-tau cases from non-tau cases, a capability which would have huge clinical and research applications.
- (2) Second objective is to provide evidence for presence of increased tau aggregation and microglial activation in FTD participants compared with controls.

To address the first question, I initially test the sensitivity of [^{18}F]AV-1451 for FTD-tau cases by examining binding in a case of FTD due to a MAPT mutation and resulting from 4 repeat tau neuropathology. I then test [^{18}F]AV-1451 specificity for FTD-tau cases by examining binding in a cohort of participants with semantic dementia (highly likely to have TDP-43 pathology) and FTD due to C9orf72 resulting from TDP-43 type B pathology, both situations where one would expect to see no increase in binding of this ‘tau radioligand’. Finally, I characterise the presence and distribution microglial activation and tau aggregation across the whole FTD spectrum and within the individual diseases, as well as assessing the relationship between tau aggregation and microglial activation in FTD.

Chapter 2: Methods

Summary

This chapter is adapted from Bevan-Jones et al, published in BMJOpen in 2015. Here I outline the methods which underpin my thesis, in particular the selection and recruitment of participants, the neuropsychological testing and imaging aspects of the protocol and an introduction of the analysis techniques which are discussed in greater detail in each chapter. This study of frontotemporal dementia is observational in nature and is conducted within the overarching Neuroimaging of Inflammation in Memory and Related Other Disorders (NIMROD) study (Bevan-Jones et al., 2017).

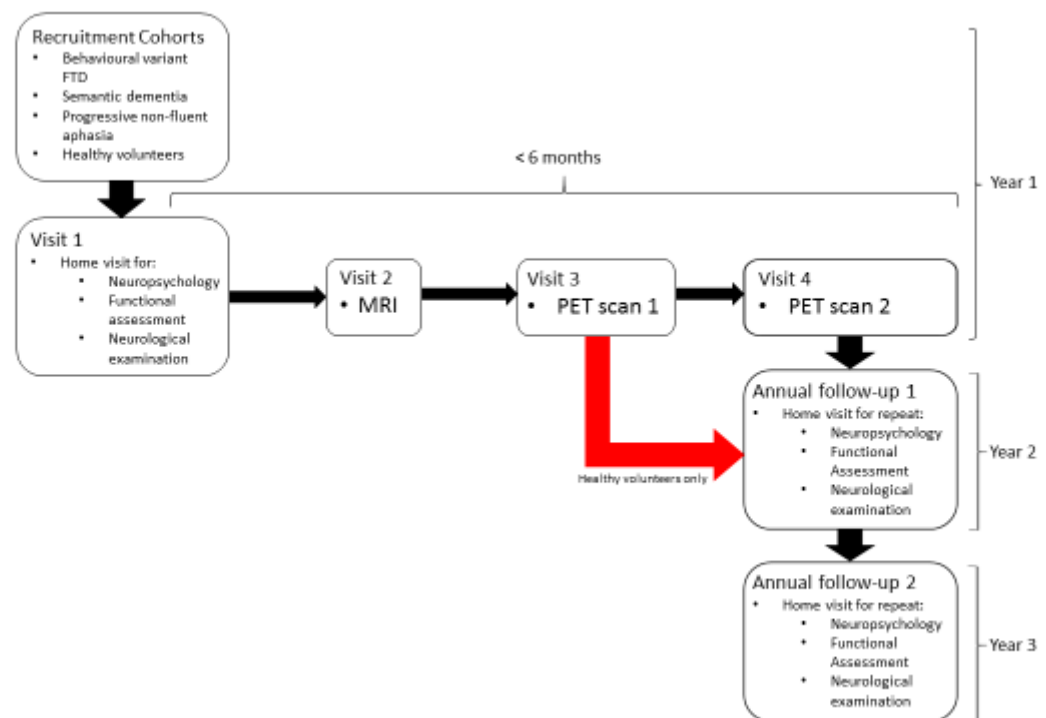


Figure 2.1: Flow chart illustrating participants' journey through the study.

Aims

The aims of this study are as follows:

- 1) To assess the sensitivity of the 'tau ligand' AV-1451 for tau pathology in FTD
- 2) To assess the specificity of the 'tau ligand' AV-1451 for tau pathology in FTD i.e. could it be used to determine tau positive cases from tau negative cases
- 3) To assess the presence of microglial activation across the FTD spectrum

Participants, Recruitment and Selection

Patient participants were recruited from cognitive disorder clinics in neurology, old age psychiatry and related services at Cambridge University Hospital (CUH) and other Trusts within the region including Cambridgeshire, Lincolnshire, Bedfordshire, Norfolk, Suffolk, Hertfordshire and Essex, where subjects are willing to travel to Cambridge for imaging studies. Case registers held by the Dementias and Neurodegeneration specialty of the UK Clinical Research Network (DeNDRoN) and the Join Dementia Research (JDR) platform (“www.joindementiaresearch.nihr.ac.uk,”) were other sources of participants. Control subjects were recruited from healthy adults within the region who have indicated a willingness to participate in dementia research via JDR or DeNDRoN. Interested, healthy friends and non-blood-related family members of patients were also recruited.

Potential participants identified as above who show willingness to take part in the research are provided with information about the study in the form of a patient information sheet. Following a period of time to consider the information, a follow-up phone call is made to inquire as to their interest in participation and to ask for further information to ensure they are eligible to take part. An appointment is then made at the study premises or at their home to provide an opportunity to ask further questions and obtain formal written consent from the participant or, in cases where the participant does not have capacity, advice from an appropriate consultee in accordance with the Mental Capacity Act 2005 (England and Wales). Consent is for participation in the study and publication of findings.

Eligibility criteria

Participants were included in the study if they were aged over 50 and had sufficient proficiency in English to allow standardised cognitive testing. All subjects except controls had a reliable informant who was able to complete questionnaires for informant related scales and provide a background history. For patient participants I included only subjects with mild to moderate dementia, as it was likely that severely impaired patient participants would be highly unlikely to comply with the study protocol. Mild to moderate dementia was defined in this study as MMSE > 12, though those with language and/or semantic impairments (in particular the semantic variant of FTD), for which the MMSE is an unsuitable screening test, were assessed using the Clinical Dementia Rating scale (Hughes, Berg, Danziger, Coben, & Martin, 1982), with a score of 2 or less indicating mild to moderate dementia.

Exclusion criteria

Potential participants were excluded if they had a concurrent major psychiatric illness (except in behavioural variant of frontotemporal dementia where this label may have been a

differential diagnosis or where psychiatric/psychotic symptoms may form part of the syndrome) or if they had a contra-indication to an MRI scan (such as a permanent pacemaker), were unable to tolerate an MRI (due to claustrophobia), or if they had a comorbidity that limits their ability to take part in the study. Potential participants were also excluded if they had atypical or focal parenchymal appearances on MRI which were not in keeping with their diagnosis. Systemic inflammatory disease was also an exclusion criterion, as was concurrent medications that might have affected study assessments (e.g. oral steroids).

Cohorts

Recruitment numbers for each of the three frontotemporal dementia cohorts were as follows: behavioural variant frontotemporal dementia 10 (only 9 had [^{11}C]PK-11195 PET), semantic dementia 11 (only 9 had [^{11}C]PK-11195 PET) and progressive non fluent aphasia 10 (all 10 had both [^{11}C]PK-11195 PET and [^{18}F]AV-1451 PET), all diagnosed according to the clinically defined international consensus diagnostic criteria laid out by Rascovsky et al.(Rascovsky et al., 2011) and Gorno-Tempini et al.(Gorno-Tempini et al., 2011). Fourteen healthy control subjects, defined as subjects with MMSE scores greater than 26 and with an absence of (i) regular memory complaints, (ii) signs or symptoms suggestive of dementia or, (iii) unstable or significant medical illnesses were also recruited to a ‘tau control’ arm (undergoing [^{18}F]AV-1451 PET) whilst fifteen healthy control participants meeting the same criteria were recruited to a ‘neuroinflammation control’ arm (undergoing [^{11}C]PK-11195 PET).

Overview of Protocol

Once written consent was provided, participants underwent neuropsychological assessment using a test battery described in detail below. The battery was tailored to participants with frontotemporal dementia. All participants also underwent an initial clinical assessment, including the collection of clinical and demographic information (including medication, smoking, alcohol, and education histories).

Participants then made between two and three visits for imaging. All participants had an MRI scan. Healthy control participants underwent one PET scan (either with [^{11}C]PK-11195 or [^{18}F]AV-1451). If possible all participants in each of the frontotemporal dementia cohorts had two PET scans ([^{11}C]PK-11195 and [^{18}F]AV-1451).

Venepuncture was carried out at the time of [^{11}C]PK-11195 imaging in all participants to measure peripheral inflammatory and other degenerative markers. Participants who provided additional consent also had a lumbar puncture for analysis of cerebrospinal fluid (CSF),

measuring both established (amyloid- β -42 and total tau) and emerging candidate biomarkers of neurodegeneration.

Each participant underwent repeat neuropsychological testing annually, for up to three years, providing a longitudinal assessment of cognitive function. Further details of each of these stages are set out below.

Initial Clinical Assessment, Neuropsychological Battery and Informant Questionnaires

At the initial visit, neuropsychological testing was undertaken using the battery of tests. For the purposes of my thesis the tests used were the Addenbrooke's Cognitive Assessment – revised edition, the frontal assessment battery, pyramids and palm trees and the Frontotemporal dementia rating scale, the key features of which are summarised in table 1. The full battery of tests undertaken as part of the NIMROD study is available in appendix 1. Clinical assessment was carried out either at the same visit or on the day of attendance for imaging. Neuropsychological follow-up using the same battery of tests was undertaken annually for up to 3 years from the date of initial assessment.

Test Name	Format	Purpose
<i>Neuropsychology Assessment</i>		
Frontal Assessment Battery (Slachevsky, 2000)	Assessment tool completed by study clinician	Assessment of frontal lobe function
Addenbrooke's Cognitive Examination – Revised (Eneida Mioshi, Dawson, Mitchell, Arnold, & Hodges, 2006)	Researcher administered structured test	Multi-domain cognitive screening tool
Pyramids and Palm Trees	Researcher administered, two alternative, forced choice, picture based test	Assessment of semantic memory
<i>Informant Questionnaires</i>		
Cambridge Behavioural Inventory (Wedderburn et al., 2008)	An 81 item carer-reported questionnaire	Assessment of several behavioural abnormalities in the everyday life including impulsivity and apathy
Frontotemporal dementia Rating Scale	A 30 item carer-reported questionnaire	Assessment of behaviour and functional abilities

Table 2.1: Neuropsychological testing within the NIMROD study for the FTD cohort

MRI Imaging

MRI scanning was performed at the WBIC using 3 Tesla Siemens scanners. For each participant a three-dimensional structural high-resolution T1 weighted sequence was performed (176 slices of 1.0 mm thickness, TE= 2.98 ms, TR = 2300 ms, flip angle =9°, acquisition matrix 256x240; voxel size = 1x1x1 mm³). All images were examined by a Consultant Radiologist at Cambridge University Hospital to exclude unexpected brain abnormalities in recruits. Participants with significant abnormalities were excluded from the study. MPRAGE T1-weighted images facilitated tissue class segmentation into grey matter, white matter and CSF respectively, and allowed non-rigid registration of standard space regions of interest, from a modified version of the Hammer's atlas that included midbrain and

cerebellum dentate (Hammers et al., 2003), to subject MRI space. Each T1 image was non-rigidly registered to the ICBM2009a template brain using ANTS (<http://www.picsl.upenn.edu/ANTS/>) and the inverse transform was applied to the Hammers atlas (resliced from MNI152 to ICBM2009a space) to bring the regions of interest to subject MRI space. The full MRI protocol for the NIMROD study is available in appendix 1.

PET Imaging

All subjects also underwent PET imaging. The radiotracers were produced at the Wolfson Brain Imaging Centre (WBIC) Radiopharmaceutical Chemistry laboratories. Both [^{11}C]PK-11195 and [^{18}F]AV-1451 were produced using the GE PETtrace cyclotron, a 16MeV proton and 8MeV deuteron accelerator. [^{11}C]PK-11195 was prepared using the “Disposable” synthesis system or GE TRACER lab FX-C module. The production of [^{18}F]AV-1451 was based on the synthetic methods developed by Avid Radiopharmaceuticals and modified to use the GE TracerLab FX-FN synthesizer at WBIC.

[^{18}F]AV-1451 PET

In summary, 370 MBq of [^{18}F]AV-1451 (high radiochemical purity (495%), specific activity of 216 +/- 60 GBq/ μmol at the end of synthesis) was injected intravenously over 30 seconds at the onset of a 90 minutes scan, with emission data subsequently reconstructed into 58 contiguous time frame images for kinetic analysis with the simplified reference tissue model (Gunn, Lammertsma, Hume, & Cunningham, 1997). PET scanning was performed on a GE Discovery 690 PET/CT. A low dose CT scan on the Discovery 690. PET pre-processing to produce non-displaceable binding potentials (BP_{ND}) for 90 brain regions was performed by in-house PET modellers (Dr Young Hong and Dr Tim Fryer). Each emission frame was reconstructed using the PROMIS 3D filtered back projection algorithm into a 128 x 128 matrix 30cm transaxial field of view, with a transaxial Hann filter cut-off at the Nyquist frequency (Kinahan & Rogers, 1989). Corrections were applied for randoms, dead time, normalization, scatter, attenuation, and sensitivity. Each emission image series was aligned using SPM8 to correct for patient motion during data acquisition (www.fil.ion.ucl.ac.uk/spm/software/spm8/). The mean aligned PET image, and hence the corresponding aligned dynamic PET image series, was rigidly registered to the T1-weighted image using SPM8 to extract values from both the Hammers atlas regions of interest and those in a reference tissue defined in the superior grey matter of the cerebellum using a 90% grey matter threshold on the grey matter probability map produced by SPM8 smoothed to PET resolution. The superior cerebellum was used as reference region as it is considered to have little or no tau pathology in frontotemporal dementia (Davies et al., 2005; Josephs et al.,

2011). All region of interest data, including the reference tissue values, were corrected for CSF partial volume effects through division with the mean region of interest probability (normalized to 1) of grey plus white matter segments, each smoothed to PET resolution. BP_{ND} was determined for each Hammers atlas region of interest using a basis function implementation of the simplified reference tissue model operating upon the Hammers atlas and reference tissue region of interest data with CSF correction (Gunn et al., 1997).

[¹¹C]PK-11195 PET

In summary, 500 MBq [¹¹C]PK-11195 (high radiochemical purity (>95%), specific activity approximately 85 GBq/μmol at end of synthesis) was injected intravenously over 30 seconds at the onset of a 75 minutes scan, with emission data subsequently reconstructed into 55 contiguous time frame images for kinetic analysis with the simplified reference tissue model (Yaqub et al., 2012). PET scanning used a GE Discovery 690 PET/CT, with attenuation correction provided by a low dose CT scan as above. The emission protocols were 75 minutes of dynamic imaging (55 frames) starting concurrently with a 500 MBq [¹¹C]PK-11195 injection. PET pre-processing to produce BP_{ND} for 90 brain regions was performed by in-house PET modellers (Dr Young Hong and Dr Tim Fryer). Each emission frame was reconstructed using the PROMIS 3-dimensional filtered back projection algorithm into a 128x128 matrix 30cm trans-axial field of view, with a trans-axial Hann filter cut-off at the Nyquist frequency (Kinahan & Rogers, 1989). Corrections were applied for randoms, dead time, normalization, scatter, attenuation, and sensitivity. Each emission image series was aligned using SPM8 to reduce the effect of patient motion during data acquisition (www.fil.ion.ucl.ac.uk). The mean aligned PET image (and hence the corresponding aligned PET image series) was rigidly registered to the T1-weighted MR image. Supervised cluster analysis was used to determine the reference tissue time-activity curve. All region of interest data were corrected for CSF contamination through division with the mean region of interest probability (normalized to 1) of grey + white matter, using SPM8 probability maps smoothed to PET resolution. BP_{ND} , a measure of specific binding, was determined for each region of interest using a basis function implementation of the simplified reference tissue model with CSF contamination correction. [¹¹C]PK-11195 BP_{ND} maps were also generated using this basis function simplified reference tissue model approach.

Kinetic modelling rather than the standardised uptake value ratio (SUVR) method was used for [¹⁸F]AV-1451 as the latter had not been validated at the start of data acquisition (Shcherbinin et al., 2016). BP_{ND} provides an estimate of the density of ligand binding sites and in any case is a more specific measure than SUVR and in older subjects with variable

perfusion, as kinetic modelling overcomes the potential problem arising from a failure to reach steady state.

As part of the NIMROD study blood samples were also taken from patients for later analysis as well as CSF samples from a sub-group. Further details of this are not included here as data did not form part of this thesis, but are available in appendix 1.

Analysis

For this study the α was set at 0.05 with a β of 0.2 giving a power of 0.8 to detect an effect size of 1 between 2 groups. Primary analysis of the PET and MRI data uses the 83 brain regions calculated in the PET pre-processing (discarding the 7 regions which consisted of CSF spaces, corpus callosum and pituitary). Using these regions of interest across the whole brain I perform parametric univariate comparisons of each diagnostic group to controls using [^{11}C]PK-11195 BP_{ND}, [^{18}F]AV-1451 BP_{ND} or grey matter volume. Comparisons are also made of specific cases against controls adjusting for small sample sizes as required (Crawford & Howell, 1998; Crawford, Garthwaite, & Crawford, 2007). Correlational analyses between ligands utilise Pearson correlations of raw BP_{ND} and of BP_{ND} adjusted for regional control mean.

Parametric and non-parametric data driven, multivariate approaches were also used to examine distribution of binding across the whole brain and for classification purposes. In particular I recurrently use non-parametric analyses of distribution in order to compare distributions of binding across cohorts blinded to absolute magnitude of binding. To describe the non-parametric analysis of distribution further, for each participant the 83 regional binding values are ranked from highest to lowest and this rank order plotted to form a single vector representative of the pattern of binding for that participant. A correlation analysis is then undertaken correlating each participant's vector with every other participant's vector. These correlations are then plotted in correlation matrices to illustrate whether the pattern of binding in one individual is similar or different from another. The matrices then form the input into another method called multidimensional scaling, in this case restricted to 2 dimensions, which plots each individual in 2 dimensions relative to the strength of correlation with every other individual in order to illustrate the groups of individuals who are the most similar. When interpreting the correlation matrices it is only necessary to consider the top row of squares (or left hand column of squares as this is its mirror image). In this row the top left square will represent the correlation of the patient against themselves and so will be perfect. Every other square to the right of this will represent the correlation of the patient with one of the controls and will be variably dissimilar. The body of the matrix to the right of the left hand column

and inferior to the top row will represent the correlation of controls with other controls. When interpreting the multidimensional scaling plots it is important to remember that they are not demonstrating correlations of 2 variables but rather spatial modes of binding according to 2 dimensions. This enables us to visualise those individuals who have similar spatial modes of binding and to see the emergence of groups. The significance of these groups is then examined in the final chapter using a cross validation linear support vector machine.

Ethics and radiation approval

The study protocol was approved by the local ethics committee, East of England - Cambridge Central Research Ethics Committee (reference: 13/EE/0104). The study is also ARSAC (Administration of Radioactive Substances Advisory Committee) approved as part of this process. The cohort is part of the MRC funded Dementias Platform UK collaboration. Approvals, consent forms and information sheets for the study are available in appendix 1.

Limitations

Although each of the following chapters includes discussion of specific limitations related to the questions each addresses, there are broader limitations which relate to the general. I briefly outline these below.

- 1) FTD is a heterogeneous entity and the diagnosis and recruitment in this study is based on clinical features and not pathologically proven.
- 2) The study, although large by FTD standards, has a small sample size which increases the chance of type II error.
- 3) Logistical problems related to availability of scanners, ligands and patients leads to variable time differences between scanning modalities and the use of two different scanners introducing a confounding source of variability and noise into the data.
- 4) The PET modelling methods used in this study reflect the available evidence when recruitment started in 2014 and so use standardised reference tissue models, in particular for AV-1451 modelling, rather than standardised uptake value ratio methods as these had not been validated.
- 5) The use of partial volume corrected data forms the basis of the following analyses which whilst accounting for the effect of atrophy in these illnesses could artificially inflate binding potentials.
- 6) The potential for off-target binding of both ligands and the difficulties in interpretation which this uncertainty causes.

Chapter 3: Sensitivity of [¹⁸F]AV-1451 to FTLD-tau pathology

Preface

The content of this chapter is predominantly the same as published in ‘Bevan Jones et al. [¹⁸F]AV-1451 PET in behavioural variant frontotemporal dementia due to MAPT mutation. *Annals of clinical and translational neurology*. 2016 Dec;3(12):940-7’. The neuropsychological assessments were performed by myself and by Robert Arnold. Pre-processing of the PET data was performed by Dr Timothy Fryer and Dr Young Hong. Analysis and interpretation was done by myself supported by Simon Jones and Dr Thomas Cope. The *post mortem* analysis in appendix 1 was contributed by Jillian Kril and Shelley Forrest. The text was written by myself and I am grateful for the input from my co-authors.

Summary

This chapter examines [¹⁸F]AV-1451 binding in the context of a microtubule associated protein tau mutation (10+16C>T), which is known to cause a genetic 4 repeat tauopathy and frontotemporal dementia, in essence assessing the sensitivity of this ligand to tau pathology in frontotemporal dementia. I demonstrate that binding is significantly abnormal in both magnitude and distribution in this non-Alzheimer’s tauopathy. These findings suggest that [¹⁸F]AV-1451 might be a useful biomarker in primary tauopathies.

Introduction

The pathogenic role of tau is well established in many neurodegenerative diseases but until recently it has only been feasible to examine the morphology, intensity and distribution of tau pathology *post mortem*. However, the recent ability to image tau aggregates *in vivo* gives us the opportunity to unpick an important aspect of the pathophysiology of neurodegeneration and how it relates to clinical disease.

There is strong evidence *in vivo* and *post mortem* that [¹⁸F]AV-1451 binds paired helical filaments of tau in Alzheimer’s disease (AD) (Marquié et al., 2015; Xia et al., 2013). The distribution and magnitude of *in vivo* tau binding correlates with AD staging (Schwarz et al., 2016), and recapitulates the anatomical distribution of focal onset forms including logopenic aphasia and posterior cortical atrophy (Ossenkoppele et al., 2016). Binding to tau in primary, non-AD tauopathies is less well established, with inconsistency between *in vivo* PET findings and *post mortem* analysis in progressive supranuclear palsy (Lowe et al., 2016; Marquié et al., 2015; Sander et al., 2016).

In order to reap the potential benefits of *in vivo* tau imaging it is important to demonstrate sensitivity of tau ligands to different types of tau pathology. Genetically determined tauopathies provide an important opportunity in this regard for validation of tau ligands outside of Alzheimer's disease. It has already been demonstrated in individuals with advanced dementia due to MAPT mutation that regional [¹⁸F]AV-1451 binding *in vivo* correlates strongly with the density of immunohistochemical tau pathology *post-mortem*, and correlates with glucose hypometabolism (Smith, Puschmann, et al., 2016), but differentiation between patients and controls has not been established, in either distribution or magnitude of [¹⁸F]AV-1451 PET. In this chapter, I compare the magnitude and distribution of [¹⁸F]AV-1451 binding in healthy controls to a patient with behavioural variant frontotemporal dementia (bvFTD) resulting from a 10+16C>T mutation in the microtubule associated protein tau gene (*MAPT*), testing the hypothesis that binding will be elevated in frontotemporal regions in this genetic tauopathy.

Methods

Family History

Proband

The patient presented aged 51 with 3 years of gradual change in behaviour and difficulty managing daily affairs, apathy, reduced empathy, obsessional behaviours, rigid routines, hyperphagia and weight gain. Examination revealed adynamic, empty speech with preserved grammar, reduced verbal fluency, anomia, semantic deficits and surface dyslexia. Eye movements were normal. Praxis, cortical sensation and visuospatial function were intact. There were no cerebellar or extrapyramidal features and no signs of anterior horn cell disease. She scored 36/100 on the Addenbrooke's Cognitive Examination (revised), and 3/26 on the frontotemporal dementia functional rating scale (Mioshi & Hornberger, 2010), indicating severe deficits. Magnetic resonance imaging revealed asymmetric, predominantly left sided, frontotemporal atrophy. She and her father had a 10+16C>T mutation of MAPT, with an H1H1 haplotype.

Father

The patient's father presented aged 59 with 10 years of insidious personality change and inappropriate behaviour without insight. He was disinhibited, restless, hyperphagic for sweet foods, with cognitive-rigidity, stereotyped behaviours and later apathy. He had semantic memory impairment, poor verbal fluency, anomia and surface dyslexia. Visuospatial function and orientation were intact. There were no ocular or motor abnormalities. His initial

Addenbrooke's Cognitive Examination score was 77/100. MRI showed frontal and anterior temporal lobe atrophy, more marked on the left. He died aged 63.

Previous neuropathological examination by the Cambridge brain bank (figure 3.1) showed moderate cerebral atrophy, most prominent in the frontal and temporal lobes, especially on the left. There was mild neuronal loss and gliosis throughout cortex, without neuritic plaques. Ballooned neurons were observed, immunopositive for phosphorylated 4-repeat tau (figure 3.1A), as well as widespread thread pathology in grey and white matter (figure 3.1B), and coiled bodies in temporal lobes (figure 3.1C). Their morphology and distribution appeared typical for sporadic frontotemporal lobar degeneration with CBD pathology (Cairns et al., 2007; Dickson et al., 2002), in keeping with MAPT mutation (Ghetti et al., 2015).

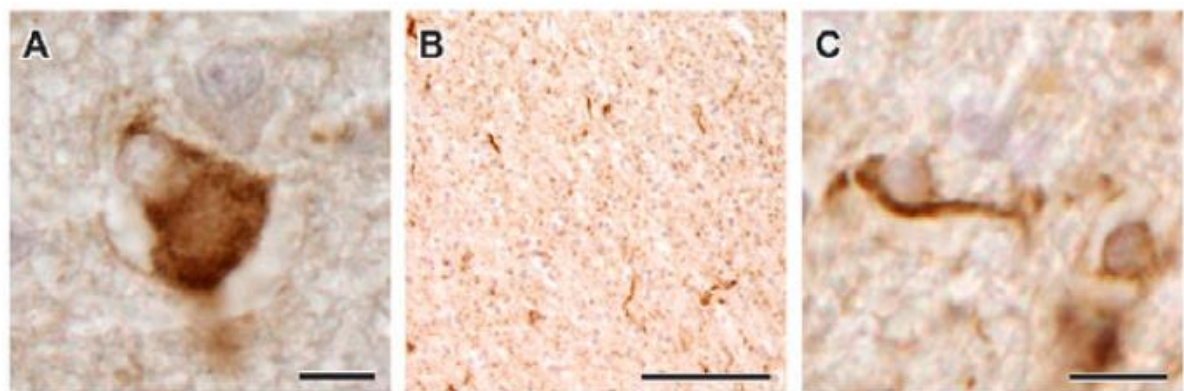


Figure 3.1: Representative neuropathological features of the proband's father's neuropathology in the superior frontal cortex showing a ballooned neuron (A), white matter threads (B), and a coiled body (C) immunostained with phosphorylated tau. Scale bar represents

Paternal grandmother

The patient's paternal grandmother developed a change in personality and behaviour, with disinhibition, hoarding and theft. Her death aged 51 was attributed to "cerebral atrophy", without neuropsychological or *post mortem* examination.

Positron emission tomography using [¹⁸F]AV-1451

PET scanning used [¹⁸F]AV-1451 and dynamic scanning over 90 minutes with a GE Advance scanner. A ⁶⁸Ge/⁶⁸Ga transmission scan enabled attenuation correction. Binding potentials, relative to a non-displaceable compartment (BP_{ND}), were determined from kinetic analysis with a simplified reference tissue model, using superior cerebellar grey-matter as the reference region. In older subjects with variable perfusion, kinetic modelling overcomes the potential problem with standardised uptake value ratios arising from a failure to reach steady

state. Brain parcellation used the Hammers brain atlas (Hammers et al., 2003), expanded to include subcortical structures (Gousias et al., 2008).

Data modelling and statistical method

The proband underwent [^{18}F]AV-1451 PET and MRI as outlined in chapter 2. Two questions were posed in the data analysis. Firstly, were there areas of the brain with higher BP_{ND} in the proband than 12 healthy adults (55-80, mean age 66, 50% male)? For each region, a robust t-score was calculated for the patient compared to the control group, adjusting for the relatively small size of the sample (Crawford & Howell, 1998). This converges with a similar Bayesian approach (Crawford et al., 2007).

Secondly, irrespective of the absolute level of ligand binding, did the distribution of binding across brain regions differ between the proband and healthy adults? An hierarchical cluster analysis approach was used. The parcellated [^{18}F]AV-1451 BP_{ND} data were converted to individual linear vectors by region of interest. These vectors were non-parametrically correlated (Spearman's rho), giving a correlation matrix (figure 3.5), and converted to a dissimilarity matrix (1-correlation, figure 3.5). Dissimilarity fed into hierarchical cluster analysis with thresholding for two groups using a complete ('farthest neighbour') method ([Http://cda.psych.uiuc.edu/multivariate_fall_2013/matlab_help/cluster_analysis.pdf](http://cda.psych.uiuc.edu/multivariate_fall_2013/matlab_help/cluster_analysis.pdf), n.d.); the most stringent linkage method for a single case (figure 3.4).

Results

Figure 3.2 contains raw maps of BP_{ND} for all individuals.

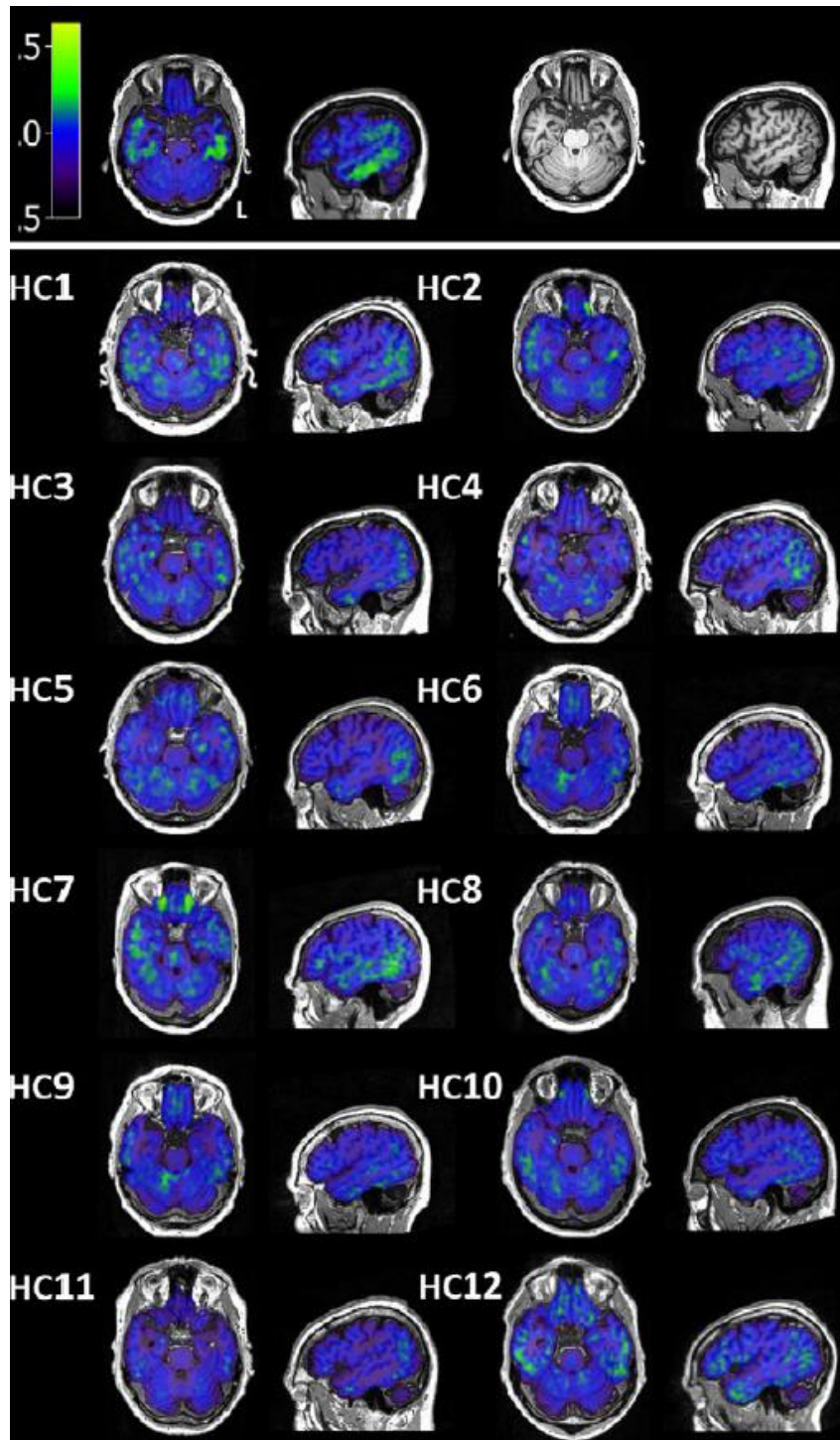


Figure 3.2: Upper panel: $[^{18}\text{F}]\text{AV-1451 BP}_{\text{ND}}$ (left) and T1-weighted MRI scan (right) for the proband. Lower panel: $[^{18}\text{F}]\text{AV-1451 BP}_{\text{ND}}$ for each individual control. $[^{18}\text{F}]\text{AV-1451 BP}_{\text{ND}}$ slices for all individuals are in the same position in native space.

Figure 3.3 illustrates the regions with significantly elevated t-scores in the proband. Bonferroni correction for 83 regions-of-interest comparisons confirmed significant differences in inferior temporal lobe, and inferior and medial temporal pole bilaterally, as well as right superior temporal pole (table 1).

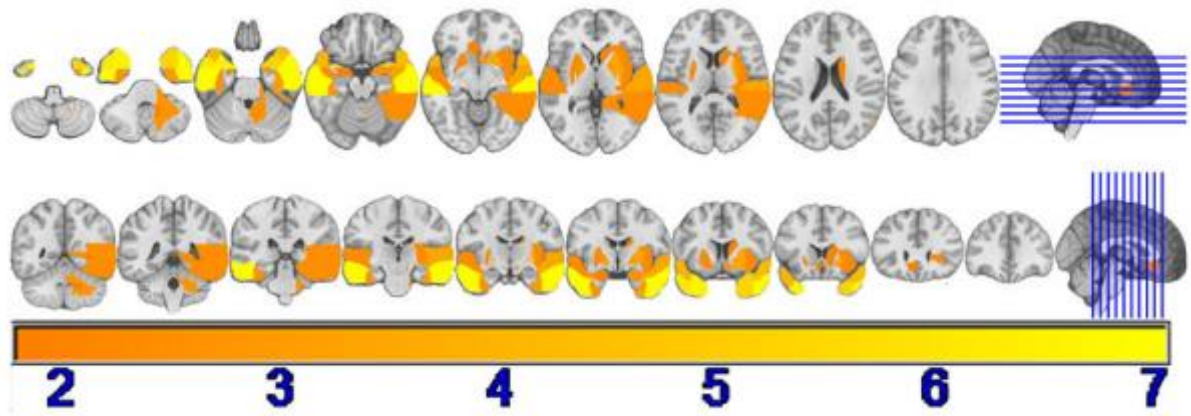


Figure 3.3: Coronal and axial sections through the template brain, overlaid with ligand binding t -scores.

As an indication of the sensitivity of the ligand in these regions, table 1 also includes a column of the maximum BP_{ND} in each region for any of the controls, as well as the mean and standard deviation across all controls. For the left inferior temporal lobe, the region with the highest t -score, the control mean BP_{ND} was 0.0086 (standard deviation 0.0346), the maximum BP_{ND} observed in any of the controls was 0.0572, and the MAPT patient's BP_{ND} was 0.2928. The MAPT patient's BP_{ND} in this region was therefore 8.2 standard deviations above the mean, and 5.8 times more unusual than any of the controls.

Hammers Atlas Brain Region	Simplified Name	Hammers Region Number	MAPT BP _{ND}	Control BP _{ND} max	Control BP _{ND} mean	Control BP _{ND} SD	Corrected t-score
'G_tem_midin_l'	Left Inferior Temporal	14	0.2928	0.0572	0.0086	0.0346	7.90
'Ant_TL_inf_lat_r'	Right Inferior Temporal Pole	7	0.5925	0.1342	0.0557	0.0738	6.99
'G_tem_midin_r'	Right Inferior Temporal	13	0.3367	0.0793	0.0140	0.0447	6.93
'Ant_TL_med_l'	Left Medial Temporal Pole	6	0.3118	0.0690	0.0094	0.0441	6.59
'Ant_TL_inf_lat_l'	Left Inferior Temporal Pole	8	0.3950	0.1245	0.0478	0.0540	6.18
'Ant_TL_med_r'	Right Medial Temporal Pole	5	0.2774	0.0860	0.0071	0.0485	5.36
'G_sup_temp_ant_r'	Right Superior Temporal Pole	83	0.4292	0.2080	0.1191	0.0705	4.23
'G_sup_temp_ant_l'	Left Superior Temporal Pole	82	0.3792	0.1995	0.0809	0.0721	3.97
'Putamen_r'	Right Putamen	39	0.4353	0.3568	0.2369	0.0552	3.45
'G_sup_temp_cent_r'	Right Superior Temporal	11	0.2122	0.1181	0.0377	0.0495	3.39
'Subcal_area_r'	Right Ventral Anterior Cingulate Cortex	79	0.3919	0.2645	0.1296	0.0751	3.35
'Pallidum_r'	Right Pallidum	43	0.5226	0.3514	0.2128	0.0995	2.99
'G_occtem_la_r'	Right Perirhinal Cortex	15	0.2112	0.1277	0.0059	0.0660	2.99
'Pallidum_l'	Left Pallidum	42	0.4468	0.3797	0.1909	0.0864	2.84
'Cerebellum_wm_r'	Right Cerebellar Grey Matter	90	-0.0009	-0.0280	-0.0614	0.0219	2.65
'G_occtem_la_l'	Left Perirhinal Cortex	16	0.1677	0.1223	0.0233	0.0531	2.61
'Amygdala_r'	Right Amygdala	3	0.2248	0.1413	0.0535	0.0636	2.59
'NuclAccumb_r'	Right Caudate	37	0.4768	0.4355	0.1970	0.1083	2.48
'PosteriorTL_r'	Right Posterior Temporal	31	0.0934	0.0625	-0.0019	0.0386	2.37
'G_sup_temp_cent_l'	Left Superior Temporal	12	0.1400	0.1135	0.0291	0.0452	2.36
'Putamen_l'	Left Putamen	38	0.3947	0.3750	0.2463	0.0617	2.31
'Insula_r'	Right Insula	21	0.1693	0.1198	0.0531	0.0493	2.26
'S_nigra_r'	Right Midbrain	75	0.4506	0.3863	0.2332	0.0958	2.18
'CaudateNucl_r'	Right Caudate	35	0.3383	0.3522	0.1738	0.0787	2.01
'Subgen_antCing_l'	Left Ventral Anterior Cingulate Cortex	76	0.1090	0.0752	-0.0107	0.0578	1.99
'Amygdala_l'	Left Amygdala	4	0.1800	0.1838	0.0514	0.0670	1.84
'Cerebellum_dentate_r'	Right Cerebellar Dentate	239	0.0850	0.0697	0.0208	0.0342	1.80
'Fl_strai_G_r'	Right Medial Orbitofrontal Cortex	53	0.2492	0.2452	0.1297	0.0646	1.78

Table 3.1: Corrected t-scores from brain regions with statistically significant ligand binding potential (BP_{ND}) at $p < 0.05$ uncorrected. Regions surviving Bonferroni correction for 83 comparisons ($p < 0.0006$) are in bold. For each region, the BP_{ND} for the for the MAPT patient is given, along with the maximum BP_{ND} observed in any of the controls and the mean and standard deviation of BP_{ND} across all controls.

Hierarchical cluster analysis of the distribution (figure 3.4) distinguished two groups. The red group contained 11 of the 12 healthy elderly individuals, while the cyan group contained the patient and 1 of the healthy individuals.

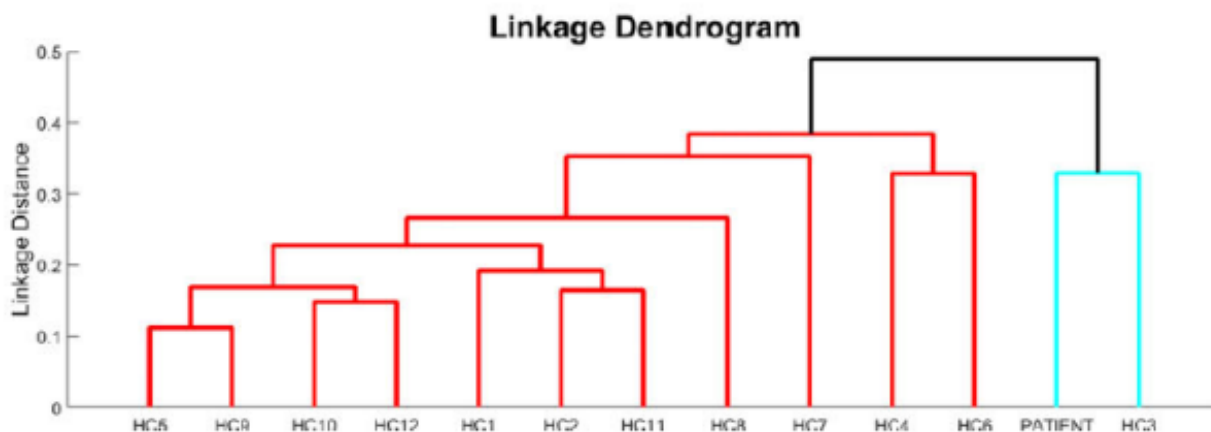


Figure 3.4: The linkage dendrogram produced by hierarchical cluster analysis. The two resultant clusters are coloured in red and cyan. Controls are numbered according to their order in the upper panel correlation and dissimilarity matrices.

Cluster analysis, blinded by non-parametric methods to the degree of ligand binding, therefore provided statistically significant classification (binomial $p=0.003$). The control classified together with the patient under the farthest neighbour method was 80 years old. She did not display any cognitive abnormalities (ACE-R 98/100, MMSE 30/30) but parametric t-test comparison of this individual to the other controls revealed higher BP_{ND} in right hippocampus ($t_{10}= 5.14$; $p<0.05$ Bonferroni corrected) and parahippocampal gyrus ($t_{10} = 4.53$; $p<0.05$ corrected) but unlike the proband, the control's BP_{ND} was not elevated in inferior temporal lobes. Therefore, although the overall pattern of regional binding in this control was less dissimilar from the MAPT patient than it was from the most dissimilar of all of the other controls, there was a clear dissociation from the MAPT case. It is possible that [¹⁸F]AV-1451 detected asymptomatic Alzheimer's disease pathology in this healthy control, as expected in a proportion of older adults. Validation linkage analysis with the 'nearest neighbour' method confirmed the patient distribution to be unique.

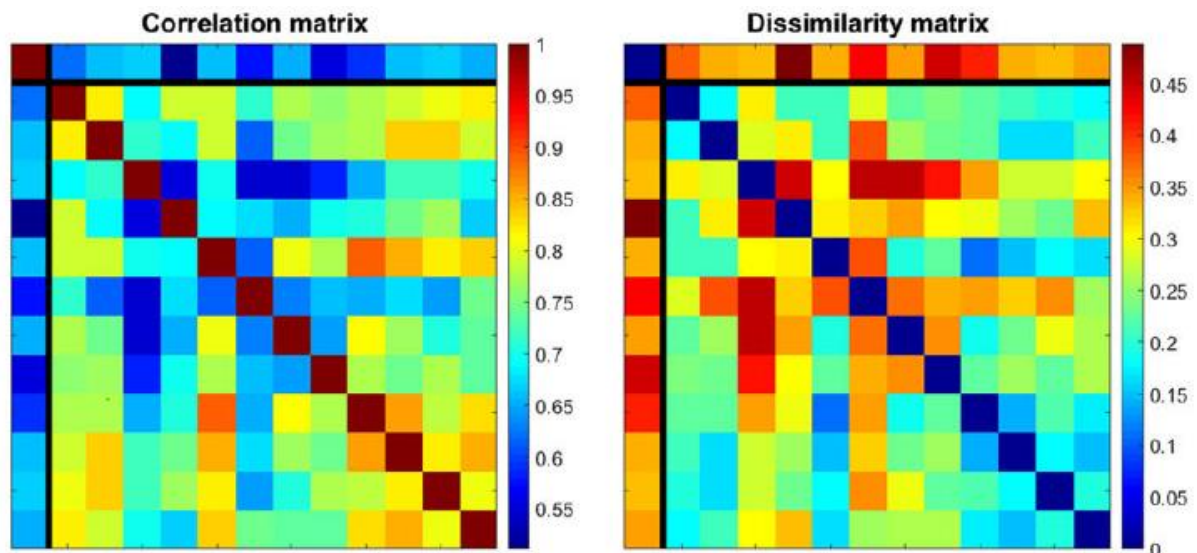


Figure 3.5: Left hand side - Spearman correlation matrix between all individuals. The first row and column, separated by black lines from the other rows and columns, represents the patient. The other twelve columns represent controls. Right hand side - The same data expressed as dissimilarities (1-correlation).

Together, these results indicate that it is not simply the case that the proband had globally elevated [^{18}F]AV-1451 BP_{ND}, but that the BP_{ND} distribution was also significantly different, reflecting regional frontotemporal lobar degeneration. The patient had particularly abnormal BP_{ND} in anterior temporal lobes and ventral anterior cingulate cortex; areas that are particularly prone to tau accumulation in frontotemporal dementia (Kertesz, Mcmonagle, Blair, Davidson, & Munoz, 2005), and that were neuropathologically most abnormal in her father.

Discussion

The main finding is of elevated [^{18}F]AV-1451 binding potential in the anterior temporal lobes and ventral anterior cingulate cortex in a patient with bvFTD due to a *MAPT* mutation, recapitulating the distribution of neuropathology expected in FTD due to a *MAPT* to mutation. The mutation in this family leads to C>T change in the *MAPT* pre-mRNA at position 16 of the splice donor site of intron 10. This results in the increased incorporation of exon 10 in *MAPT* mRNA, creating an accumulation of the 4-repeat tau isoform which neuropathologically resembles the pathology of corticobasal degeneration (Pickering-Brown et al., 2004). This finding therefore provides evidence for the sensitivity of [^{18}F]AV-1451 to non-Alzheimer's tauopathy.

Given the clinicopathological heterogeneity of FTD, replication and further validation of tau tracers such as [^{18}F]AV-1451 in this setting would have major implications for diagnosis and clinical trials in disorders associated with frontotemporal lobar degeneration. PET studies in

cases with *MAPT* mutations provide an important facet of such validation, especially in combination with a strong, clear history and the availability of neuropathological confirmation. It is also the case that morphology and biochemistry of tau pathology varies with different *MAPT* mutations and the expectation is that the sensitivity of [¹⁸F]AV-1451 to these variations in tau pathology will be significant given the difference in magnitude of binding in Alzheimer's disease compared to this case.

The primary limitation of this report is that it is of one participant with a *MAPT* mutation. It also does not address the specificity of binding of [¹⁸F]AV-1451, which will be addressed in the next chapter which assesses [¹⁸F]AV-1451 binding in syndromes associated with TDP-43 deposition. Whilst one control was linked with the patient in the hierarchical cluster analysis, this may be accounted for by the fact that she was an outlier of the control group in age and is perhaps more likely to harbour asymptomatic Alzheimer's pathology.

In conclusion, the intensity and distribution of binding of the tau ligand [¹⁸F]AV-1451 in a patient with a *MAPT* 10+16C>T mutation supports the use of this ligand in clinical studies of dementia, including frontotemporal lobar degeneration and corticobasal syndrome.

Chapter 4: Specificity of [¹⁸F]AV-1451 in FTLD – binding in Semantic dementia

Preface

The content of this chapter is predominantly the same as published in ‘Bevan-Jones et al. [¹⁸F] AV-1451 binding *in vivo* mirrors the expected distribution of TDP-43 pathology in the semantic variant of primary progressive aphasia. J Neurol Neurosurg Psychiatry. 2018 Oct 1;89(10):1032-7.’. The neuropsychological assessment was performed by myself and by Robert Arnold. Pre-processing of the PET data was performed by Dr Timothy Fryer and Dr Young Hong. Analysis and interpretation was done by myself supported by Simon Jones and Dr Thomas Cope. The text was written by myself and I am grateful for the input from my co-authors.

Summary

Semantic dementia, including the semantic variant of primary progressive aphasia (svPPA), is strongly associated with TDP-43 type C pathology. It provides a useful model in which to test the specificity of *in vivo* binding of the putative tau ligand [¹⁸F]AV-1451, which is elevated in frontotemporal lobar degeneration tauopathies as demonstrated in the previous chapter. In this chapter I examine [¹⁸F]AV-1451 binding in seven patients (five with svPPA, two with ‘right’ semantic dementia). Two independent pre-processing methods were used and both demonstrate clearly elevated binding potential (BP_{ND}) in temporal lobes, lateralising according to their clinical syndrome. [¹⁸F]AV-1451 binding *in vivo* in regions that are likely to contain TDP-43 and not significant tau pathology suggests a non-tau target for [¹⁸F]AV-1451 and question the specificity of this ligand for tau pathology in frontotemporal dementia.

Introduction

The importance of biomarkers in neurodegenerative disorders is well established. Positron emission tomography (PET) has played an important role in biomarker development, illustrated by the impact of the Pittsburgh Compound B on both research and clinical practice in Alzheimer’s disease (Klunk et al., 2004). Whilst β-amyloid is central to the neuropathology of Alzheimer’s disease, in the vast majority of cases of frontotemporal lobar degeneration the pathology is characterised by misfolding and aggregation of either tau (~40%) or TDP-43 (~50%), with fewer cases of fused in sarcoma (FUS) pathology (<10%) (Mackenzie et al., 2009, 2011; MacKenzie et al., 2010). A major aim for clinical research and drug development has been the development of biomarkers that enable pathological

classification and longitudinal assessment *in vivo*, with quantitative and qualitative differentiation of neurodegenerative syndromes based on their underlying proteinopathy: tau vs TDP-43 vs β -amyloid.

The radioligand [^{18}F]AV-1451 was developed from compound screening in Alzheimer's brains and is selective for tau *versus* β -amyloid and *versus* α -synuclein (Xia et al., 2013). *In vitro* and *in vivo* studies have confirmed that [^{18}F]AV-1451 binding in Alzheimer's disease correlates strongly with phenotypic variation (Ossenkoppele et al., 2016), clinical severity and Braak stage (Johnson et al., 2016; Scholl et al., 2016; Schwarz et al., 2016). There are fewer studies in frontotemporal lobar degeneration, but [^{18}F]AV-1451 has also been shown to be sensitive *in vivo* to frontotemporal dementia associated with mutation of the microtubule associated protein tau gene (MAPT) (Bevan-Jones et al., 2016; Smith, Puschmann, et al., 2016; Spina et al., 2017), and in progressive supranuclear palsy (Passamonti et al., 2017). There is also evidence of modest binding to primary tauopathies in *post mortem* studies (Sander et al., 2016). However, the specificity of [^{18}F] AV-1451 for tau pathology remains controversial.

Here, I test the properties of [^{18}F]AV-1451 in semantic dementia, including the semantic variant of primary progressive aphasia (svPPA) and its non-dominant homologue, right semantic dementia (R-SD). Both syndromes display TDP-43 pathology in 75-90% of cases (John R Hodges et al., 2010; Spinelli et al., 2017), with tau pathology rarely present at *post mortem*. I test two complementary hypotheses:

- 1) In clinically diagnosed semantic dementia (likely TDP-43 pathology) the non-displaceable binding potential (BP_{ND}) of [^{18}F]AV-1451 is not increased compared with healthy older adults.
- 2) Independent of absolute levels of [^{18}F]AV-1451 BP_{ND} , the distribution of binding across brain regions is similar in patients and healthy controls.

Case Number	Demographics	Symptom duration	Presenting Clinical features	ACE-R	FAB	PPT	Diagnosis
L1	71, Male, Left handed, 15 years education	8 years	Anomia Impaired single word comprehension Surface dyslexia Obsessional behaviour	43	11	25	SvPPA
L2	69, Male, Right handed, 9 years education	7 years	Anomia Impaired single word comprehension Surface dyslexia Inability to use previously familiar tools	9	3	0	SvPPA
R3	59, Male, Right handed, 14 years education	6 years	Rigid obsessional behaviour Reduced empathy Prosopagnosia Anomia Impaired single word comprehension Surface dyslexia	79	15	48	R-SD
R4	68, Male, Right handed, 16 years education	5 years	Rigid obsessional behaviour Reduced empathy Prosopagnosia Anomia Impaired single word comprehension Surface dyslexia	77	11	28	R-SD
L5	64, Female, Right handed, 14 years education	4 years	Anomia Impaired single word comprehension Surface dyslexia Prosopagnosia Concrete thinking	72	16	44	SvPPA
L6	66, Male, Left handed, 17 years education	4 years	Anomia Impaired single word comprehension Surface dyslexia Withdrawn behaviour Prosopagnosia	71	18	48	SvPPA
L7	64, Male, Right handed, 13 years education	6 years	Anomia Impaired single word comprehension Surface dyslexia Mild prosopagnosia	68	15	47	SvPPA
Twelve Controls	Age: 65.5 (range 55-74, sd 7.1) Sex: 6:6 Education years: 15.8 (range 11-19, sd 2.1)	Not applicable	Not applicable	95.3 (range 89-99, sd 3.2)	-	-	-

Table 4.1: Demographic, clinical, neuropsychological [Addenbrooke's Cognitive Examination – revised (ACE-R), Frontal Assessment Battery (FAB), and Pyramids and Palm trees (PPT)] and diagnosis for each participant with semantic dementia and for the group of 12 controls.

Methods

MRI and PET imaging

Each participant underwent T1-weighted MRI (3T Siemens Trio or 3T Siemens Prisma, MPRAGE sequence, 1mm isotropic voxels) before PET. Manufacture of [^{18}F]AV-1451 used synthetic methods developed by Avid Radiopharmaceuticals, modified for GE TracerLab FX-FN synthesizer at the Wolfson Brain Imaging Centre, Cambridge. A GE Discovery TM 690 PET/CT scanner was used. 370 MBq of [^{18}F]AV-1451 was injected over 30 seconds at the onset of a 90 minutes scan. Emission data were reconstructed in 58 contiguous time frame images. Each emission frame was reconstructed using the PROMIS 3D filtered back projection algorithm into a 128 x 128 matrix 30cm transaxial field of view, with a transaxial Hann filter cut-off at the Nyquist frequency (Kinahan & Rogers, 1989). Corrections were applied for randoms, dead time, normalization, scatter, attenuation, and sensitivity. Each emission image series was aligned to correct for patient motion during data acquisition (www.fil.ion.ucl.ac.uk/spm/software/spm8). The BP_{ND} was determined by kinetic modelling with a simplified reference tissue model. The reference tissue was defined in the superior grey matter of the cerebellum, using a 90% grey matter threshold on the grey matter probability map produced by SPM8, smoothed to the PET resolution. The superior cerebellum was chosen as a reference region as it is unlikely to contain substantial pathology in semantic dementia (0 out of 15 cases of semantic dementia in the Cambridge brain bank had cerebellar pathology).

Two independent pre-processing methods were evaluated. (i) The data were co-registered with T1-weighted images. Regions of interest were defined by cortical parcellation and modified subcortical segmentation using the Desikan-Killiany atlas in the PetSurfer toolbox within Freesurfer (Greve et al., 2016, 2014). The BP_{ND} values in each region were partial volume corrected using the Symmetric Geometric Transfer Matrix method (Sattarivand, Kusano, Poon, & Caldwell, 2012). (ii) the mean aligned PET image, and hence the corresponding aligned dynamic PET image series, was rigidly registered to the T1-weighted image using SPM8, so as to extract values from the Hammersmith Atlas n30r83 (<http://brain-development.org/brain-atlases>) modified with brain stem and cerebellar parcellation. All region of interest data, including the reference tissue values, were corrected for the cerebrospinal fluid fraction through division with the mean region of interest probability (normalized to 1) of grey plus white matter segments, each smoothed to PET resolution.

Analysis

To test Hypothesis 1, I used general linear models with t-tests for each region of interest, excluding extraparenchymal regions, first comparing each patient to the control group, as a case series [c.f. (Bevan-Jones et al., 2016)]. I then compared the patients group-wise to the controls. Data were corrected for multiple comparisons to control the false discovery rate at $q < 0.05$ (Benjamini & Hochberg, 1995; Genovese, Lazar, & Nichols, 2002).

To test Hypothesis 2, regional binding potentials for each subject were converted to a linear vector. Spearman's rank order method was used to perform non-parametric correlation with all other subjects, creating a correlation 'similarity' matrix of the distribution of BP_{ND} , disregarding its absolute intensity. The inverse of this matrix (the 'dissimilarity' matrix) formed the input to multi-dimensional scaling and hierarchical cluster analysis. The performance of both farthest neighbour and average linkage methods was assessed (c.f. Passamonti *et al.*, 2017).

Results

Figure 4.1 shows axial and sagittal views of the T1 weighted images for each participant with semantic dementia, confirming the severe asymmetric temporal polar atrophy in svPPA and R-SD respectively. Also shown are the uncorrected raw $[^{18}F]AV-1451$ BP_{ND} maps for each individual patient and a representative control. Statistical comparisons against controls using method (i) are shown for each patient individually and for the group. All patients had significant elevation of BP_{ND} in temporal lobes compared to controls, and in all but one individual this survived correction for multiple comparisons across the whole brain. As a group, for method (i), significant elevation of BP_{ND} at FDR $q < 0.05$ was observed in the following regions of the left hemisphere: superior, middle and inferior temporal lobe, insula cortex, fusiform gyrus, temporal banks and accumbens. In the right hemisphere: amygdala, caudate and superior temporal cortex. For method (ii), the following regions showed significant elevation of BP_{ND} at FDR $q < 0.05$: In the left hemisphere: medial anterior and lateral anterior temporal lobe, superior, middle and inferior temporal gyri, fusiform gyrus, insula, thalamus and nucleus accumbens. In the right hemisphere: lateral anterior temporal lobe, middle and inferior temporal gyrus, medial anterior temporal lobe.

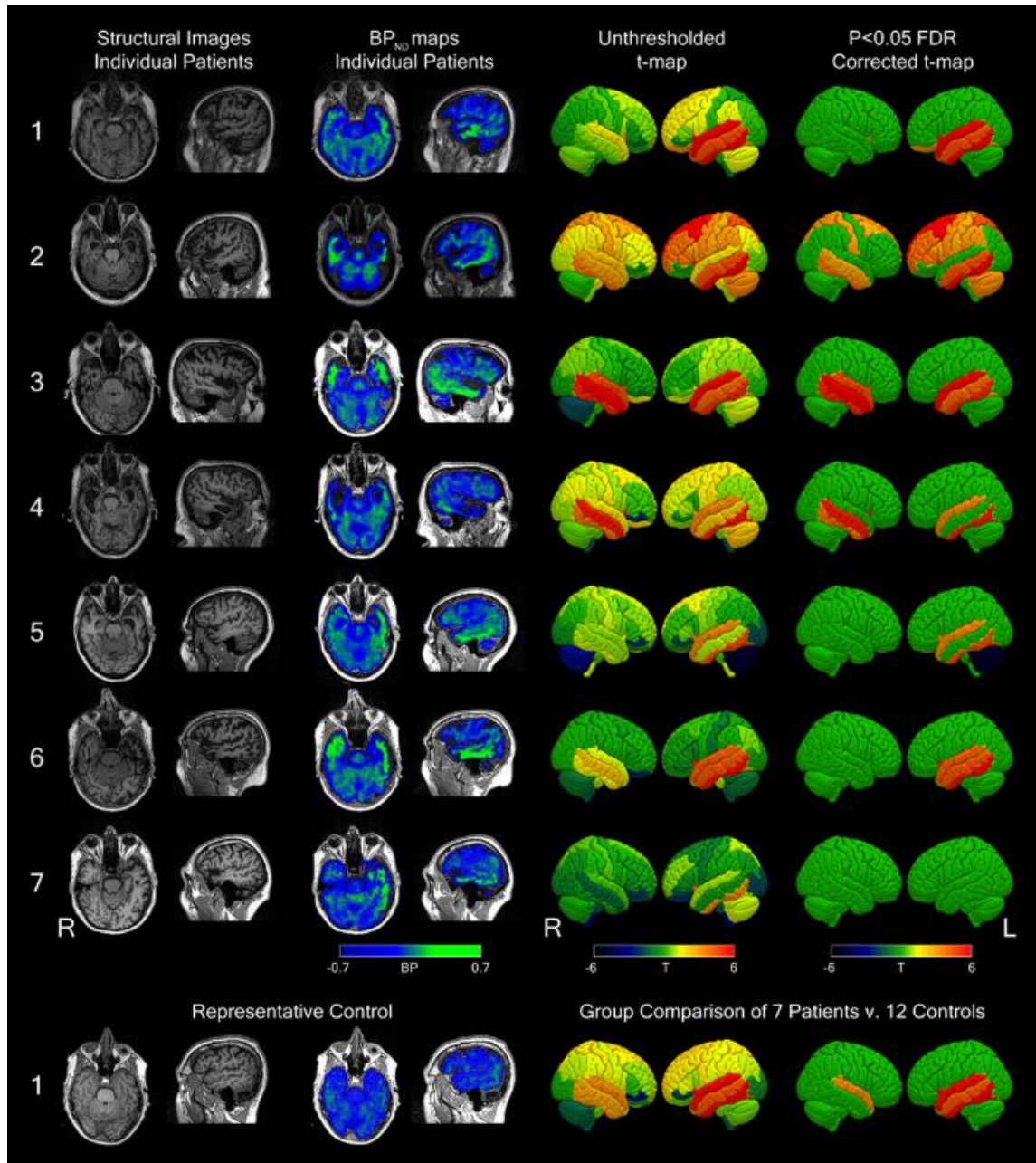


Figure 4.1: Using data pre-processed by extra method (i). Upper panel – column 1: axial and sagittal views of T1-weighted images for each semantic dementia participant, column 2: raw axial and sagittal BPND maps for each patient, column 3: unthresholded surface-rendered regional T-maps for each subject against all controls, column 4: equivalent T-maps thresholded at $p < 0.05$, corrected for false discovery rate. Lower panel – T1-weighted images and BPND maps for a representative control and the group comparisons of all 7 patients vs 12 controls both uncorrected (column 3) and corrected for false discovery rate $p < 0.05$ (column 4). The numbering of individual patients is consistent with figure 4.2 and table 4.1.

Non-parametric multidimensional scaling of BP_{ND} distribution clearly separated patients from controls (figure 4.2). With either pre-processing method, hierarchical cluster analysis of these data detected SD with 86% sensitivity and 100% specificity. Cluster analysis, blinded by non-parametric methods to the degree of ligand binding, therefore provided statistically significant unsupervised classification (Yates corrected χ^2 (1, $N = 19$) = 11.3, $p = 0.0008$). Identical results were obtained with both average and farthest neighbour linkage methods. The patient

misclassified as a control had mild and relatively early disease and lay between the control and patient distributions on multidimensional scaling (figure 4.2, case L7).

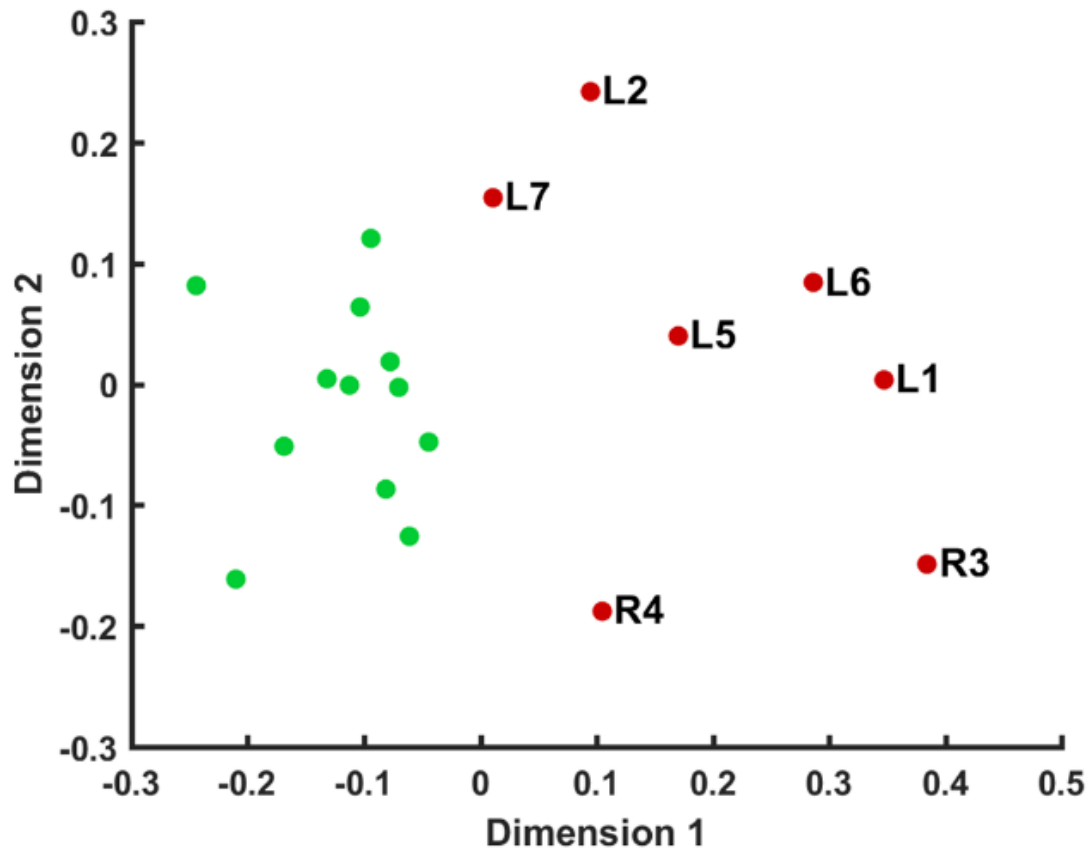


Figure 4.2: Two dimensional scaling of non-parametric whole brain regional correlations using data pre-processed by the extra method. Red dots represent patients, and green dots controls. Patient labels correspond to semantic dementia participants in table 1.

Discussion

I present evidence for consistently elevated [^{18}F]AV-1451 BP_{ND} in seven cases with either svPPA or its non-dominant right hemisphere homologue, R-SD. Contrary to the null hypotheses, in all seven cases individually and at the group level, the regions known to be most affected by TDP-43 pathology (Cairns et al., 2007; Keith A. Josephs et al., 2011) demonstrated increased [^{18}F]AV-1451 binding compared to controls. This did not merely reflect a global increase in BP_{ND}, as six of the seven cases were correctly classified by the relative *distribution* of BP_{ND} across the brain. The significant regional binding to likely TDP-43 pathology in svPPA and R-SD indicates that this ligand is not selective for tau, and casts doubt on the utility of [^{18}F]AV-1451 to sub-type frontotemporal dementias according to tau vs. TDP-43 pathology. While this suggests that the use of [^{18}F]AV-1451 to select tau-mediated frontotemporal dementia populations for clinical trials is unlikely to be effective, the

ligand may still retain a potential role in the longitudinal assessment of the degree and distribution of pathological burden across the frontotemporal dementia spectrum.

Semantic dementia in the form of svPPA represents a highly stereotyped and well defined clinical syndrome with characteristic structural neuroimaging (Hodges et al., 1992; Snowden et al., 1989; Warrington, 1975). Its non-dominant homologue displays similar features, but typically with a later presentation, more behavioural disturbance and prosopagnosia (Chan et al., 2009). Within the spectrum of frontotemporal dementias, both svPPA and R-SD show very strong clinicopathological correlations with TDP-43 pathology, especially type C (Cairns et al., 2007; Hodges et al., 2010; Josephs et al., 2011; Mackenzie et al., 2011; Snowden et al., 2007). This contrasts with the behavioural variant of frontotemporal dementia which can result from either TDP-43 or tau pathology with approximately equal likelihood.

Although the majority of semantic dementia cases have TDP-43 pathology, a small minority of cases may arise from tauopathies, most often Alzheimer's disease pathology or Pick's disease pathology (Hodges et al., 2010; Snowden et al., 2007; Spinelli et al., 2017). In keeping with this, 25 of 34 patients clinically diagnosed with semantic dementia (or svPPA) and in the Cambridge Brain Bank are positive for Ubiquitin or TDP-43 and negative for both tau and β -amyloid. Similarly, the recent report of *post mortem* examination in clinical cases of svPPA at four centres in North America (Spinelli et al., 2017) confirmed that the majority (24/29) had TDP-43 pathology (mainly type C). The five who did not have TDP-43 pathology exhibited clinical features out of keeping with the classical syndrome of svPPA, such as short disease duration and extrapyramidal features. These features are not present in any of the individuals I report. Although I do not have autopsy confirmation of the cases reported here, four out of seven participants have negative biomarkers for Alzheimer's pathology and, overall, it is highly unlikely that the explanation for our findings is that all individuals in this study had a primary or secondary tauopathy.

Whilst the *in vivo* and *post mortem* studies of [^{18}F]AV-1451 in Alzheimer's disease are compelling, the binding characteristics of [^{18}F]AV-1451 in non-AD tauopathies remain controversial. *In vivo* studies have been encouraging, with significant binding demonstrated in frontotemporal dementia due to mutations in the Microtubule associated protein tau (MAPT) gene (Bevan-Jones et al., 2016; Smith, Puschmann, et al., 2016; Spina et al., 2017) and in progressive supranuclear palsy (Passamonti et al., 2017). This contrasts with reports from *post mortem* studies, which predominantly describe low level binding to the tau aggregates of frontotemporal lobar degeneration (Lowe et al., 2016; Marquie et al., 2015; Sander et al., 2016). These *post mortem* studies make it increasingly clear that the primary, tertiary and

quaternary structures of tau, as well as the type and maturity of tau pathology (Lowe et al., 2016), are important determinants of [¹⁸F]AV-1451 binding. This implies that the predominantly straight filaments of 4 repeat tau that constitute the pathology of progressive supranuclear palsy and corticobasal degeneration, and the 3 repeat tau of intraneuronal Pick bodies, lead to less intense binding than that seen in Alzheimer's pathology, with its balanced 3-/4-repeat tauopathy in the form of paired helical filaments.

The discordance between *in vivo* and *post mortem* findings led to the proposal of 'off target' binding sites. In the last two years several possibilities have been hypothesised, particularly to explain the characteristic pattern of basal ganglia binding seen in almost all participants. These include neuromelanin (Hansen et al., 2016; Marquié et al., 2015), iron, calcium, and Biondi ring tangles (Lowe et al., 2016). None of these potential targets is anatomically compatible with the pattern of cortical binding seen here, which is in a distribution expected for pathology in semantic dementia (Cairns et al., 2007; Snowden et al., 2007). One plausible explanation for the elevated signal observed here could be spill out from increased binding in white matter, for example to the expression of monoamine oxidase B by reactive astrocytes. The intrinsic resolution of PET, combined with the degree of atrophy in the semantic dementia cohort, makes it very difficult to distinguish binding in white or grey matter. However, there is a paucity of evidence for [¹⁸F]AV-1451 binding to monoamine oxidase B, unlike other tau radioligands such as the THK compounds. The possibility of binding to monoamine oxidase B was explored early in the development of [¹⁸F]AV-1451 (Chien, Bahri, Szardenings, Walsh, & Mu, 2013; Xia et al., 2013) and its favourable profile in this regard was important in its progress to clinical studies.

Another possibility is that [¹⁸F]AV-1451 binds to very low levels of abnormal tau that have been reported to co-exist with TDP-43 in some cases [25], to proteins associated with cellular stress in TDP-43 pathologies, or to some other cellular marker of neurodegeneration. Alternatively, it could be that the *in vivo* binding demonstrated here mirrors the *post mortem* binding of [¹⁸F]AV-1451 to TDP-43 type C [14,34], despite the low level or absent binding to most TDP-43 pathology [14,34]. Overall, I retain an open mind as to the identity of the non-tau proteins and cell types to which [¹⁸F]AV-1451 is binding in Semantic Dementia.

One must also consider some caveats in the analytical methods of the imaging pipelines. In particular, the extreme regional atrophy of SD complicates normalisation and PET analysis, including modelling decisions such as partial volume correction, which is necessary in order to prevent significant binding being obscured by the degree of atrophy. Mis-registration errors arising from extreme atrophy may also influence PET estimates. However, these

considerations are unlikely to account for these findings for two main reasons. Firstly, the bright signal of elevated [^{18}F]AV-1451 binding is visible in the temporal lobes of uncorrected BP_{ND} maps in all single subjects in native space (figure 4.1). Secondly, highly similar patterns of significant binding are seen with two independent methods of data pre-processing, using different tissue segmentation and correction methods and parcellation with different brain atlases. The use of an appropriate reference tissue region may also be complicated in neurodegenerative diseases. In particular, there is emerging evidence that specific patterns of atrophy occur in the cerebellum across a range of disorders (Gellersen et al., 2017). In frontotemporal dementia, cerebellar atrophy and pathology are well described in cases of the behavioural variant, and particularly in cases resulting from expansions in C9orf72 (Whitwell et al., 2012). However, in semantic dementia cerebellar atrophy has not been described, and the typical distribution of TDP-43 type C pathology does not involve the cerebellum (Davies et al., 2005). We have examined fifteen cases of semantic dementia in the Cambridge brain bank, and in no case was cerebellar pathology found. Additional reassurance that our results are not driven by a possible group difference in cerebellar pathology comes from our hierarchical cluster analysis. This non-parametric analysis of the distribution of pathology across the whole brain is blind to absolute BP_{ND} values; the effect of a group difference in a reference region would be to change the overall level of binding across the brain, without modifying the relative distribution of pathology. The fact that I was able to recover the group structure with 86% sensitivity and 100% specificity argues against cerebellar pathology being a significant driver of our findings.

Validation of the specificity of [^{18}F]AV-1451 binding in both Alzheimer's disease and frontotemporal lobar degeneration is highly important. Binding in the presence of neurodegeneration without tauopathy poses serious questions for both clinical and research applications of this ligand, although it may nonetheless be useful to evaluate the progression of neurodegenerative diseases and normal ageing. The magnitude of elevations in binding potential were similar to those previously observed in MAPT mutation (Bevan-Jones et al., 2016) and Progressive Supranuclear Palsy, but lower than those observed in Alzheimer's disease (Passamonti et al., 2017). While this study did not directly compare FTD-TDP (in semantic dementia) to FTD-tau cases, the lack of selectivity of [^{18}F]AV-1451 for tauopathies challenges the utility of this ligand for pathological differentiation *in vivo*. Determining the binding site or sites will be important as, even if this is not specific to tau aggregation, it may provide valuable insights into the cellular mechanisms of neurodegeneration, perhaps in regions that are yet to display volume loss or hypometabolism. In order to determine the best

use of this ligand, full characterisation of the behaviour of [^{18}F]AV-1451 in frontotemporal lobar degeneration with longitudinal imaging and *post mortem* validation is essential.

Chapter 5: Specificity of [¹⁸F]AV-1451 in FTLTD – binding in C9orf72 FTD

Preface

The content of this chapter is predominantly the same as published in ‘Bevan-Jones et al. [¹⁸F] AV-1451 binding is increased in frontotemporal dementia due to C9orf72 expansion. *Annals of Clinical and Translational Neurology*. 2018 Oct;5(10):1292-6.’. The neuropsychological assessment was performed by myself and by Robert Arnold. Pre-processing of the PET data was performed by Dr Timothy Fryer and Dr Young Hong. Analysis and interpretation was done by myself supported by Simon Jones and Dr Thomas Cope. The text was written by myself and I am grateful for the input from my co-authors.

Summary

In this chapter I assessed [¹⁸F]AV-1451 binding in behavioural variant frontotemporal dementia due to a hexanucleotide repeat expansion in C9orf72, characterised by TDP-43 pathology. This builds on findings in the previous chapter of increased binding in patients with semantic dementia (where patients are presumed to have TDP-43 pathology) and tests whether this is the case in a genetic form of frontotemporal dementia where TDP-43 is known definitively to be the pathology. Answering this question, I show that the C9orf72 mutation increases binding in frontotemporal cortex, with a distinctive distribution of binding compared with healthy controls.

Introduction

[¹⁸F]AV-1451 was developed as a specific marker of paired helical filament tau (PHF-tau) pathology in Alzheimer’s disease (AD), and is selective for PHF-tau over β -amyloid and α -synuclein *in vitro* (Xia et al., 2013). *In vivo* studies have confirmed elevated and distributed binding in keeping with typical and atypical presentations of Alzheimer’s disease (Ossenkoppele et al., 2016; Scholl et al., 2017), consistent with Braak staging (Schwarz et al., 2016). Binding characteristics in neurodegenerative diseases other than Alzheimer’s disease are controversial.

In vivo and *post mortem* studies indicate increased [¹⁸F]AV-1451 binding in patients with frontotemporal dementia due to mutations in the microtubule associated protein tau (MAPT), albeit with differences between mutations associated with paired helical filament tauopathy and straight filament tauopathy (Bevan-Jones et al., 2016; Smith, Ohlsson, et al., 2016). Regionally elevated binding also occurs in another tauopathy, progressive supranuclear palsy

(Cope et al., 2018; Passamonti et al., 2017). However, [^{18}F]AV-1451 binding is also seen in patients with semantic dementia, a disease strongly associated with TAR DNA binding Protein-43 (TDP-43), especially type C (Bevan-Jones et al., 2017; Makaretz et al., 2017). The non-tau target of [^{18}F]AV-1451 binding in semantic dementia is unknown and, whilst *post mortem* studies suggest a lack of binding to TDP-43 pathology (Lowe et al., 2016; Marquié et al., 2015; Sander et al., 2016), the elevated binding seen *in vivo* is in keeping with the characteristic anatomical distribution of neuropathology in semantic dementia.

A limitation of previous studies of [^{18}F]AV-1451 binding in semantic dementia cases is their reliance on clinico-pathological correlations to interpret the neuroimaging data. In this report, I describe [^{18}F]AV-1451 binding in a patient with a clear familial case of frontotemporal dementia (FTD) due to a hexanucleotide repeat expansion in the C9orf72 gene. This is the commonest genetic cause of FTD (Bang et al., 2015) and it is strongly associated with TDP-43 pathology (Mackenzie et al., 2011) without the presence of any tau. I examine not only the magnitude of the [^{18}F]AV-1451 binding, but also the pattern of regional distribution across cortical and sub-cortical structures.

Case and controls

A 53 year old man presented with 3 year history of progressive behavioural change characterised by disinhibition, grandiosity, and stereotyped behaviours. He subsequently developed semantic impairments and anomia, with loss of single word comprehension and surface dyslexia. At the time of imaging, he scored 53/100 on the Addenbrooke's Cognitive Examination – revised edition (ACE-R, reference range >88) and 25/30 on the Mini Mental State Examination (MMSE). There were significant carer endorsements for changes in memory, challenging behaviours, altered eating habits and abnormal beliefs on the revised Cambridge Behavioural Inventory. Structural magnetic resonance imaging of his brain confirmed frontotemporal atrophy. His maternal uncle had died of motor neurone disease. Genetic testing confirmed a hexanucleotide repeat expansion in C9orf72. He met diagnostic criteria for definite behavioural variant frontotemporal dementia (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). He underwent research structural MRI and [^{18}F]AV-1451 positron emission tomography (PET) as part of the Neuroimaging of Inflammation in Memory and Related Other Disorders (NIMROD) study (W Richard Bevan-Jones et al., 2017)(Passamonti et al., 2017). Thirteen healthy volunteers acted as controls (age range 55-80, 6 Male, ACE-R range 89-99, MMSE range 28-30) and underwent the same neuroimaging and behavioural protocol.

Data modelling and statistical method

When comparing the case with the C9orf72 genetic expansion and controls, two questions were posed. First, were there regions of the brain with increased non-displaceable binding potential (BP_{ND})? Secondly, irrespective of the absolute level of ligand binding, did the distribution of binding across brain regions differ? This second question focusses on the multivariate distribution or pattern of binding, in relation to the distribution of neuropathological substrates of frontotemporal dementias. The pattern may be abnormal, even where no single region on its own has particularly high binding of [¹⁸F]AV-1451.

To address the first question, the brain was parcellated into 83 regions using the Hammersmith atlas n30r83 modified to include some additional subcortical structures (Passamonti et al., 2017). The [¹⁸F]AV-1451 BP_{ND} in each region was calculated using the methods described in Passamonti et al (Passamonti et al., 2017) to obtain regional BP_{ND} which were adjusted for cerebrospinal fluid (CSF) partial volume effects. Individual t-tests were then performed in each region to compare the observed [¹⁸F]AV-1451 BP_{ND} in the C9orf72 case and controls, correcting for multiple comparisons across the full data range (FDR correction, $p < 0.05$, plus illustration at the more liberal threshold of $p < 0.05$ uncorrected).

To address the second question, a hierarchical cluster analysis was used. The CSF corrected and parcellated [¹⁸F]AV-1451 BP_{ND} data were then converted to individual linear vectors containing all regions of interest. The pairwise Spearman's rank order correlations were calculated between all subjects, and the inverse taken to produce a dissimilarity matrix. This represents the difference in [¹⁸F]AV-1451 BP_{ND} distribution between every pair of subjects, blinded to the absolute level of that binding. These dissimilarity measures were used to calculate a linkage dendrogram using an average distance method.

I also compared regional grey matter volume between the case and control group. Regional grey matter volumes were calculated during PET processing and parcellated using the same atlas. For each region, linear models were used with region volume, age and total intracranial volume as covariates (O'Brien et al., 2012). T-tests were performed between the residual of the patient's volume and predicted volume, based on the controls' regression, and the control residuals. The p-values for each test were increased to compensate for the degrees of freedom used in the control regression. False discovery rate (FDR) correction was then applied to these adjusted p-values in the same manner as the [¹⁸F]AV-1451 BP_{ND}.

Results

[¹⁸F]AV-1451 in C9orf72 FTD versus controls

T1 weighted MPRAGE images are shown in figure 5.1A and raw BP_{ND} images uncorrected for partial volume effects in figure 5.1B. The left frontotemporal regions demonstrated increased BP_{ND} in the C9orf72 case compared to controls. After correcting for multiple comparisons ($p < 0.05$ FDR), significant abnormalities were observed in left fusiform gyrus, left medial anterior temporal lobe, left middle and inferior temporal gyri, and left lateral inferior temporal lobe (figure 5.1E). In addition to this, more liberal thresholding without correction for multiple comparisons demonstrated bilateral changes, with elevated BP_{ND} also found in the right medial anterior temporal lobe, left anterior superior temporal gyrus, superior and middle frontal gyri, pallidum, and substantia nigra (figure 5.1D).

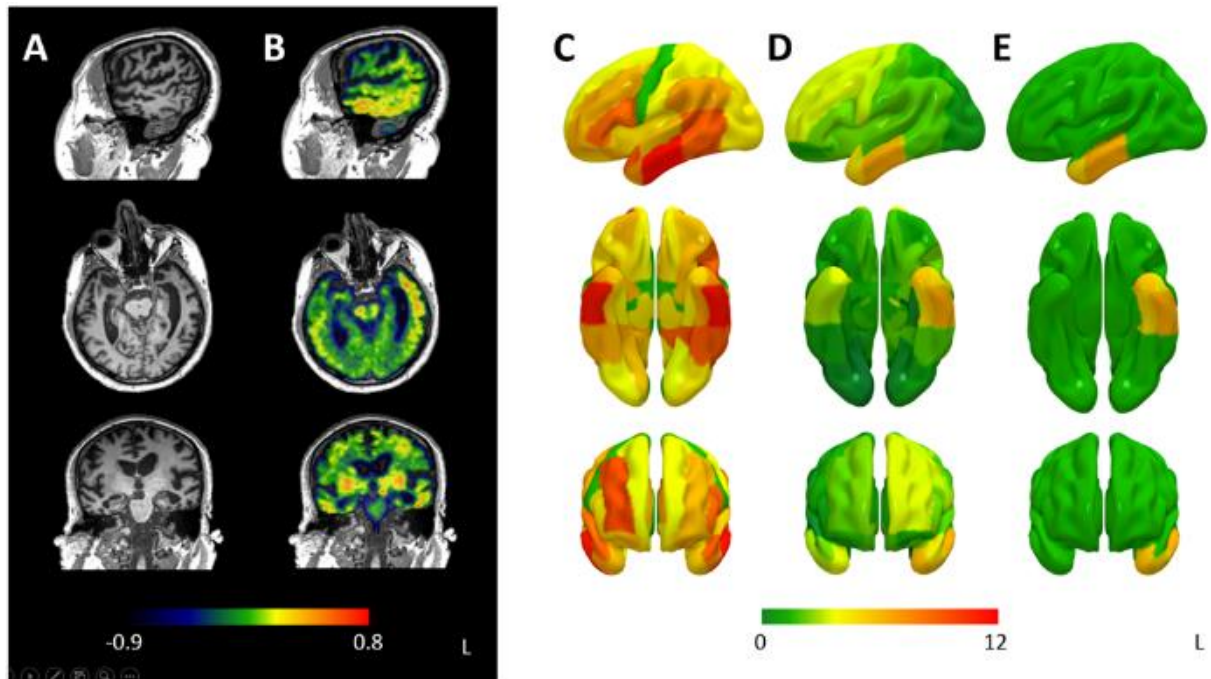


Figure 5.1: Panels A shows selected sagittal, coronal and axial slices from the structural MRI (T1 weighted MPRAGE) of the C9orf72 case. Panel B shows the [¹⁸F]AV-1451 binding potential (BP_{ND}) without correction for partial volume effects in the same planes. Panel C shows the unthresholded t-scores for grey matter atrophy on a volumetric rendering on the smoothed MNI152 template MRI. Panel D shows unthresholded t-scores for (BP_{ND}) in the same way. Panel E shows the same data but thresholded at $p < 0.05$ corrected for false discovery rate.

The distribution of BP_{ND} was also clearly dissimilar in the C9orf72 case compared to all controls (upper panel of figure 5.2). The hierarchical clustering analysis indeed classified the patient as an outlier compared to controls (lower panel of figure 5.2).

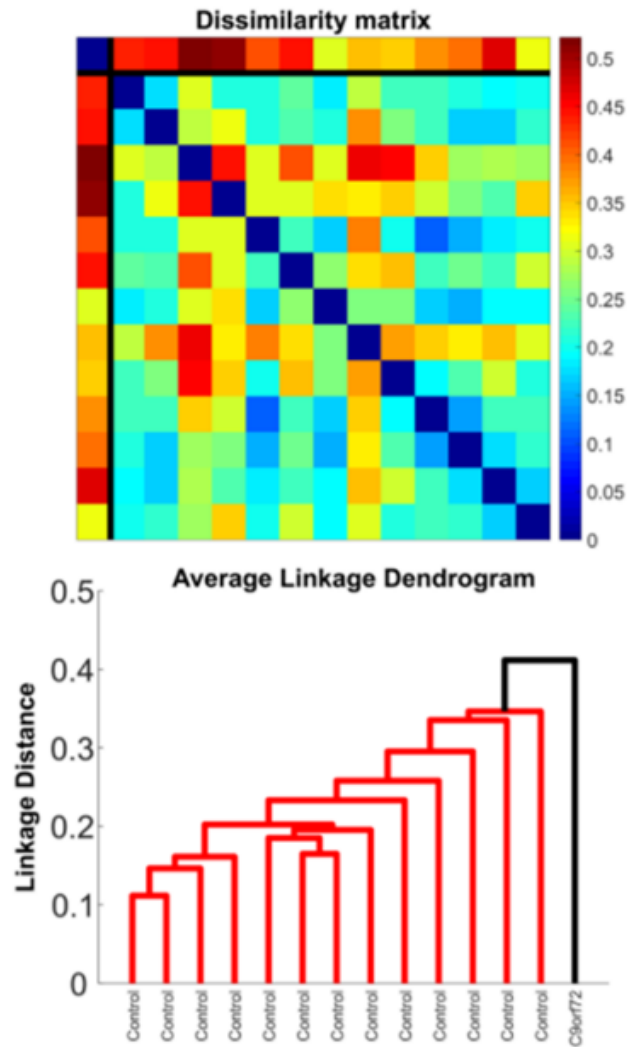


Figure 5.2: Upper panel: Spearman dissimilarity matrix (1-correlation) between all individuals (C9orf72 FTD case and 12 controls). The first row and column, separated by black lines from the other rows and columns, represents the patient. The other thirteen columns represent controls. Lower panel: the average linkage dendrogram produced by hierarchical cluster analysis. The two resultant clusters are coloured in red and black.

The regional volume analysis revealed widespread grey matter loss, most marked in the temporal lobes, which remained after FDR correction (figure 5.1C). T-scores along with FDR corrected p-values for both [^{18}F]AV-1451 and grey matter volume are shown in table 5.1 below.

Region name	AV T score	AV FDR corrected p value	Atrophy T score	Volume FDR corrected p value
Left middle and inferior temporal gyrus	5.98	0.01	11.40	0.000
Left anterior temporal lobe medial part	5.19	0.01	4.59	0.002
Left fusiform gyrus	4.20	0.03	6.24	0.001
Left anterior temporal lobe lateral part	3.95	0.04	6.75	0.000
Left superior temporal gyrus anterior part	3.60	0.06	8.61	0.000
Left pallidum	2.83	0.17	0.02	0.987
Left substantia nigra	2.78	0.17	2.07	0.078
Right anterior temporal lobe medial part	2.58	0.22	5.25	0.001
Left superior frontal gyrus	2.44	0.25	2.75	0.029
Left middle frontal gyrus	2.40	0.25	5.62	0.001
Left precentral gyrus	1.99	0.42	2.26	0.059
Right anterior temporal lobe lateral part	1.83	0.46	6.18	0.001
Right middle and inferior temporal gyrus	1.70	0.51	11.27	0.000
Right fusiform gyrus	1.68	0.51	4.61	0.002
Right presubgenual frontal cortex	1.62	0.51	4.10	0.004
Left cerebellum dentate	1.54	0.52	3.08	0.018
Left putamen	1.35	0.64	2.24	0.059
Left gyrus cinguli posterior part	1.26	0.67	3.37	0.011
Right superior frontal gyrus	1.23	0.67	2.55	0.038
Left inferior frontal gyrus	1.11	0.74	8.30	0.000
Right superior temporal gyrus anterior part	1.08	0.74	8.62	0.000
Right substantia nigra	1.05	0.74	1.86	0.109
Left insula	1.05	0.74	4.51	0.003
Left amygdala	1.04	0.74	4.96	0.001
Left subgenual frontal cortex	0.93	0.77	2.66	0.033
Right nucleus accumbens	0.91	0.77	0.16	0.887
Left posterior orbital gyrus	0.90	0.77	5.61	0.001
Right middle frontal gyrus	0.87	0.77	9.21	0.000
Right putamen	0.85	0.77	1.11	0.325
Left inferiolateral remainder of parietal lobe	0.82	0.77	6.23	0.001
Left thalamus	0.80	0.77	4.07	0.004
Left superior temporal gyrus posterior part	0.77	0.79	5.06	0.001
Left posterior temporal lobe	0.74	0.80	8.69	0.000
Left postcentral gyrus	0.70	0.81	3.06	0.018
Right medial orbital gyrus	0.66	0.83	3.55	0.009
Right subgenual frontal	0.64	0.83	2.26	0.059

cortex				
Left medial orbital gyrus	0.59	0.87	3.49	0.010
Left nucleus accumbens	0.52	0.89	0.36	0.745
Right pallidum	0.36	0.95	1.27	0.259
Left caudate nucleus	0.33	0.95	2.56	0.038
Right straight gyrus	0.33	0.95	2.83	0.026
Right thalamus	0.32	0.95	3.60	0.008
Right caudate nucleus	0.32	0.95	0.65	0.567
Right anterior orbital gyrus	0.27	0.95	5.29	0.001
Right amygdala	0.24	0.95	5.51	0.001
Left straight gyrus	0.20	0.96	4.53	0.003
Left lateral orbital gyrus	0.16	0.97	5.20	0.001
Bilateral brainstem pons	0.11	0.99	5.19	0.001
Right cerebellum grey matter	0.08	0.99	4.83	0.002
Right gyrus cinguli posterior part	0.07	0.99	3.42	0.011
Left cingulate gyrus anterior part	0.06	0.99	6.12	0.001
Right lateral orbital gyrus	0.05	0.99	3.92	0.005
Left superior parietal gyrus	-0.02	0.99	2.64	0.034
Left cerebellum grey matter	-0.02	0.99	5.29	0.001
Right subcallosal area	-0.03	0.99	0.71	0.540
Left presubgenual frontal cortex	-0.17	0.97	2.43	0.045
Right precentral gyrus	-0.25	0.95	1.28	0.259
Bilateral brainstem midbrain	-0.25	0.95	3.76	0.007
Left anterior orbital gyrus	-0.28	0.95	5.09	0.001
Right parahippocampal and ambient gyri	-0.37	0.95	2.83	0.026
Right superior parietal gyrus	-0.38	0.95	4.06	0.004
Right insula	-0.39	0.95	5.63	0.001
Right inferior frontal gyrus	-0.48	0.92	4.00	0.005
Right posterior orbital gyrus	-0.52	0.89	4.06	0.004
Right cingulate gyrus anterior part	-0.52	0.89	5.38	0.001
Right posterior temporal lobe	-0.81	0.77	5.82	0.001
Left lateral remainder of occipital lobe	-0.87	0.77	3.84	0.006
Left subcallosal area	-0.89	0.77	5.42	0.001
Right postcentral gyrus	-0.91	0.77	5.94	0.001
Right inferiolateral remainder of parietal lobe	-1.15	0.73	5.61	0.001
Bilateral brainstem medulla	-1.23	0.67	4.17	0.004
Left parahippocampal and ambient gyri	-1.26	0.67	3.05	0.018
Right superior temporal gyrus posterior part	-1.44	0.58	7.81	0.000
Left lingual gyrus	-1.57	0.52	2.53	0.039

Right lateral remainder of occipital lobe	-1.57	0.52	2.79	0.027
Right cerebellum dentate	-1.63	0.51	1.97	0.092
Right hippocampus	-1.82	0.46	1.38	0.229
Right cuneus	-1.95	0.42	0.69	0.546
Right lingual gyrus	-1.96	0.42	5.07	0.001
Left cuneus	-2.16	0.36	0.63	0.571
Left hippocampus	-3.16	0.11	5.66	0.001

Table 5.1: T-scores and FDR corrected p-values for [^{18}F]AV-1451 binding potential and for atrophy in each region, ordered by magnitude of [^{18}F]AV-1451 binding potential T-score.

Discussion

C9orf72 expansions are strongly associated with TDP-43 type B and dipeptide repeat pathology. I therefore infer that at 53 years old, with the C9orf72 expansion, and a classical FTD presentation, the present case has TDP-43 pathology without tauopathy. Despite this, the participant exhibited elevated [^{18}F]AV-1451 binding in frontotemporal regions of a magnitude greater than the 95th centile of binding in controls but within the interquartile range for binding in Alzheimer's disease (Passamonti et al., 2017) and semantic dementia (Bevan-Jones et al., 2017) (figure 5.3). Combined with similar findings in 14 cases with semantic dementia (Bevan-Jones et al., 2017; Makaretz et al., 2017), this suggests that the [^{18}F]AV-1451 PET ligand is not specific for tau over TDP-43 pathology in Frontotemporal lobar degeneration. Therefore, while [^{18}F]AV-1451 might retain a role in tracking disease by visualising the distribution and/or severity of neuropathology in a person with frontotemporal dementia, it is unlikely to have utility for cohort selection for disease modifying trials that target tau or TDP-43 specific disease mechanisms.

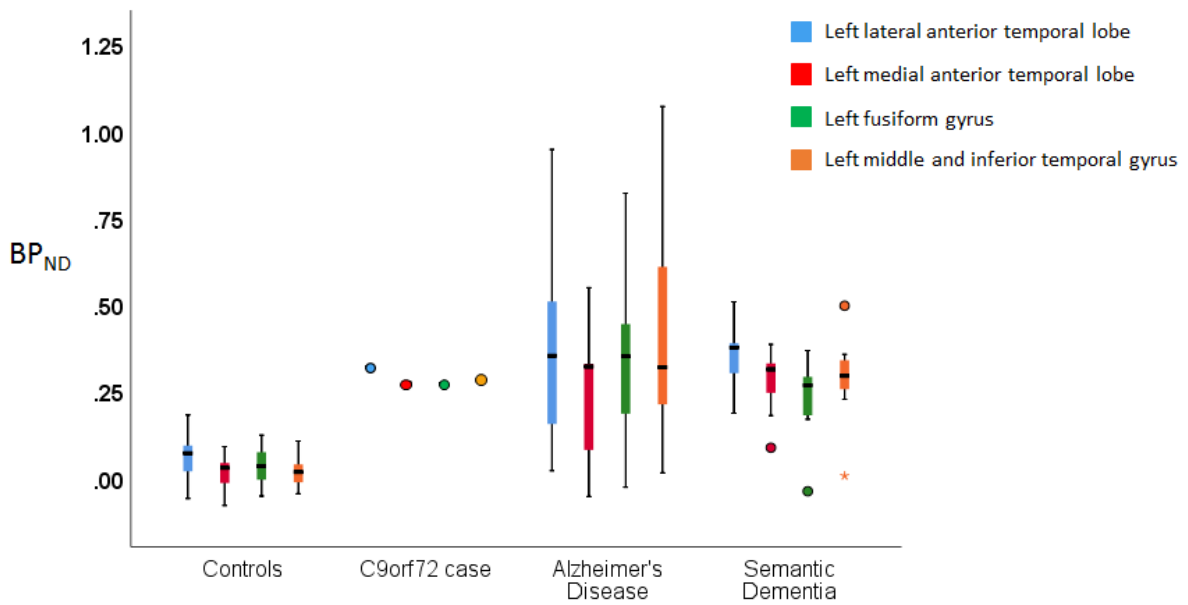


Figure 5.3: Boxplots of the non-displaceable [^{18}F]AV-1451 binding potential in those regions of interest that are significantly elevated in the C9orf72 case compared to controls after FDR correction. As well as data for the C9orf72 case and the control population, comparative data are shown using the same analysis methods for our previously published cohorts with Alzheimer's disease and Semantic Dementia.

As expected in this participant with moderate dementia from a C9orf72 expansion, there was widespread grey matter loss with a predilection for frontotemporal regions, and particularly temporal regions in keeping with his clinical syndrome. The regions of elevated BP_{ND} with largest t-scores were situated within the atrophic regions but elevated BP_{ND} did not occur across all atrophic regions. The colocation of elevated BP_{ND} and atrophy is perhaps unsurprising if [^{18}F]AV-1451 is binding to a non-tau molecule associated with C9orf72-related neurodegeneration.

There are limitations in this study. First I have analysed data from a single case against a control group. Larger studies in C9orf72 FTD might expand upon these results, as C9orf72 frontotemporal dementia is clinically and neuroanatomically heterogeneous (Devenney et al., 2014). While the C9orf72 case is young, mutation positive, and meets diagnostic criteria for definite behavioural variant frontotemporal dementia, I lack biomarker or pathology proof of the absence of coincidental Alzheimer tau pathology that may occur in a small percentage of adults in their fifties. However, age-related pre-symptomatic Alzheimer pathology is rendered an unlikely explanation for the data, both by the low population prevalence at 53, and the regions found to have elevated binding in the case which are clearly distinct from those usually observed in Alzheimer's disease. Finally, I note that the calculation of [^{18}F]AV-1451 BP_{ND} data uses the superior cerebellar grey matter as a reference region, as in my other recent papers (Bevan-Jones et al., 2016; Bevan-Jones et al., 2017; Passamonti et al., 2017).

Although across most of the genetic and sporadic forms of FTD this region remains unaffected, in previous cases with C9orf72 expansion, cerebellar atrophy and dipeptide aggregation have been described (Mackenzie et al., 2013). In mitigation of this potential shortcoming, whilst as expected there was cerebellar grey matter atrophy in the present C9orf72, there was no observable elevated cerebellar [^{18}F]AV-1451 signal in the uncorrected PET BP_{ND} map. Moreover, if elevated binding in this reference cerebellar region were present, it would have only reduced the estimated BP_{ND} elsewhere, making the risk of false negative results more likely. In any case, it is important to note that the level of cerebellar binding does not affect the non-parametric analysis of distributional binding differences.

Elevated binding in genetic TDP-43 associated FTD provides further evidence for anatomically specific binding of [^{18}F]AV-1451 to non-tau targets. However, the sensitivity to longitudinal changes in TDP-43 associated FTD, and the molecular identity of the [^{18}F]AV-1451 target, remain to be determined.

Chapter 6: Neuroinflammation and protein aggregation in the early stages of FTD pathogenesis

Preface

The content of this chapter is predominantly the same as published in ‘Bevan-Jones et al. ‘*In vivo* evidence for pre-symptomatic neuroinflammation in a MAPT mutation carrier. *Annals of Clinical and Translational Neurology*. 2018’. The neuropsychological assessment was performed by myself and by Robert Arnold. Pre-processing of the PET data was performed by Dr Timothy Fryer and Dr Young Hong. Analysis and interpretation was done by myself supported by Simon Jones and Dr Thomas Cope. The text was written by myself and I am grateful for the input from my co-authors.

Summary

Neuroinflammation occurs in frontotemporal dementia, however its timing relative to protein aggregation and neuronal loss is unknown. In this chapter I quantify these processes in a pre-symptomatic carrier of the 10+16 MAPT mutation I show microglial activation in frontotemporal regions, despite a lack of protein aggregation or atrophy in these areas. The distribution of microglial activation better discriminated the carrier from controls than did protein aggregation at this pre-symptomatic disease stage. These findings suggest an early role for microglial activation in frontotemporal dementia.

Introduction

Genetic aetiologies account for up to a third of cases of frontotemporal dementia (FTD) (Bang et al., 2015), and may provide important insights into the pathophysiology of sporadic FTD (Forrest et al., 2018; Josephs, 2018). Whilst disease arising from genetic abnormalities in microtubule associated protein tau (MAPT) accounts for 5-10% of cases, around 40% of patients with FTD display tau pathology (Bang et al., 2015). Pre-symptomatic studies of familial FTD have shown cognitive, structural, and network functional connectivity changes preceding symptomatic onset of FTD by several years (Cash et al., 2018; Rohrer et al., 2015). The study of pre-symptomatic mutation carriers with other modalities such as positron emission tomography (PET) may yield insights into early pathophysiological processes (Forrest et al., 2018; Josephs, 2018).

Here, I focus on two key, potentially modifiable processes, neuroinflammation and tau protein aggregation. Neuroinflammation has been implicated in FTD by cerebrospinal fluid (Sjogren et al., 2004), genetic (Baker et al., 2006; Broce et al., 2018; Rayaprolu et al., 2013) and PET

studies (Cagnin et al., 2004). Microglial activation may alter brain homeostasis in protective or deleterious manners through inflammatory pathways, cytotoxicity, and changes in neuronal plasticity (Perry, Nicoll, & Holmes, 2010). Despite evidence of neuroinflammation in FTD, little is known about their *in vivo* relationship to protein aggregation and neuronal loss.

Neuroinflammation and tau pathology can be quantified using PET. [^{11}C]PK-11195 is a biomarker of activated microglia and therefore a surrogate measure for neuroinflammation (Banati, 2002b). [^{18}F]AV-1451 binds preferentially to paired helical filament tau neuropathology (Marquie et al., 2015; Xia et al., 2013) but is also sensitive to the straight filament tauopathy present in the 10+16 MAPT mutations in familial FTD (Bevan-Jones et al., 2016). I use both ligands and structural magnetic resonance imaging (MRI) in a pre-symptomatic carrier of the 10+16 MAPT mutation, associated with a straight filament 4 repeat tauopathy, to test the hypothesis that neuroinflammation occurs before detectable tau aggregation and brain atrophy, even when the primary driver of the disease must be associated with tau following the MAPT mutation.

Methods

A 53 year old female pre-symptomatic carrier of a 10+16 MAPT mutation underwent neuropsychological testing, structural MRI, [^{11}C]PK-11195 PET, and [^{18}F]AV-1451 PET within a two-month period. Blood was taken for analysis of C-reactive protein (CRP), a non-specific peripheral marker of inflammation, at the time of [^{11}C]PK-11195 PET. A first-degree relative with the same MAPT mutation had previously been diagnosed with behavioural variant FTD.

For each PET ligand two questions were posed. Firstly, were there areas of the brain with higher BP_{ND} in the MAPT carrier compared to the control group? For each region, a t-score was calculated compared to the control group. Secondly, irrespective of the absolute level of ligand binding, did the distribution of binding across brain regions differ between the MAPT carrier and the control group? For each ligand the parcellated data were converted to individual linear vectors of regions of interest. These vectors were non-parametrically correlated (Spearman's rho), resulting in a correlation matrix, which was converted to a dissimilarity matrix, and fed into hierarchical cluster analysis based on average linkage distance. The information provided by these ligands was compared by assessing the non-parametric correlation of their distributions in the MAPT carrier and controls separately.

Finally, I compared regional grey matter volumes in the 83 regions which had been calculated from the T1-weighted MPRAGE images as part of the PET pre-processing method and

parcellated using the same atlas. A general linear model was applied to each region independently to look for differences between the MAPT carrier and [¹⁸F]AV-1451 control group including age and total intracranial volume as predictors of no interest.

Data from all three modalities were also compared to an individual with established MAPT behavioural variant FTD, whose full background and [¹⁸F]AV-1451 results have previously been published (Bevan-Jones et al., 2016).

Results

Demographics and neuropsychological scores for the MAPT carrier and controls are summarised in table 6.1.

	MAPT carrier	AV-1451 controls	PK-11195 controls
Age	53	67 (55-80)	70 (59-84)
Gender (F:M)	Female	7:6	8:7
Education (years)	11	16 (11-19)	14 (10-19)
Addenbrooke's cognitive examination - revised (out of 100)	86	95 (89-99)	93 (79-100)
Frontal Assessment Battery (out of 18)	16	n/a	n/a
FTD rating scale (%)	80	n/a	n/a

Table 6.1: Demographic information and neuropsychological test scores for the MAPT mutation carrier and the two control groups.

Raw BP_{ND} maps for both $[^{11}C]PK-11195$ and $[^{18}F]AV-1451$ alongside T1-weighted MPAGE images for the MAPT carrier are displayed in figure 6.1.

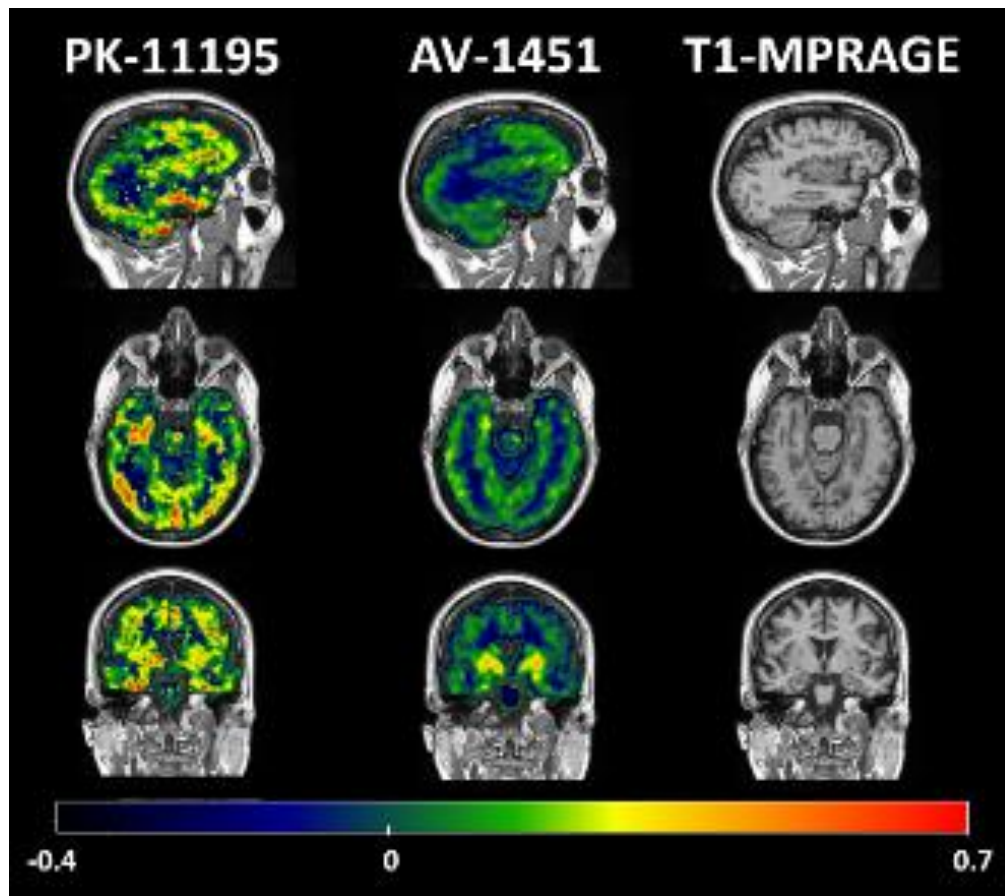


Figure 6.1: Sagittal, axial and coronal slices of the raw BP_{ND} maps for $[^{11}C]PK-11195$, $[^{18}F]AV-1495$ and T1-weighted MPAGE in the MAPT carrier. The BP_{ND} scale bar runs along the bottom of the figure.

Un-thresholded t-scores for [^{11}C]PK-11195 and [^{18}F]AV-1451 are shown in figure 6.2 for display purposes.

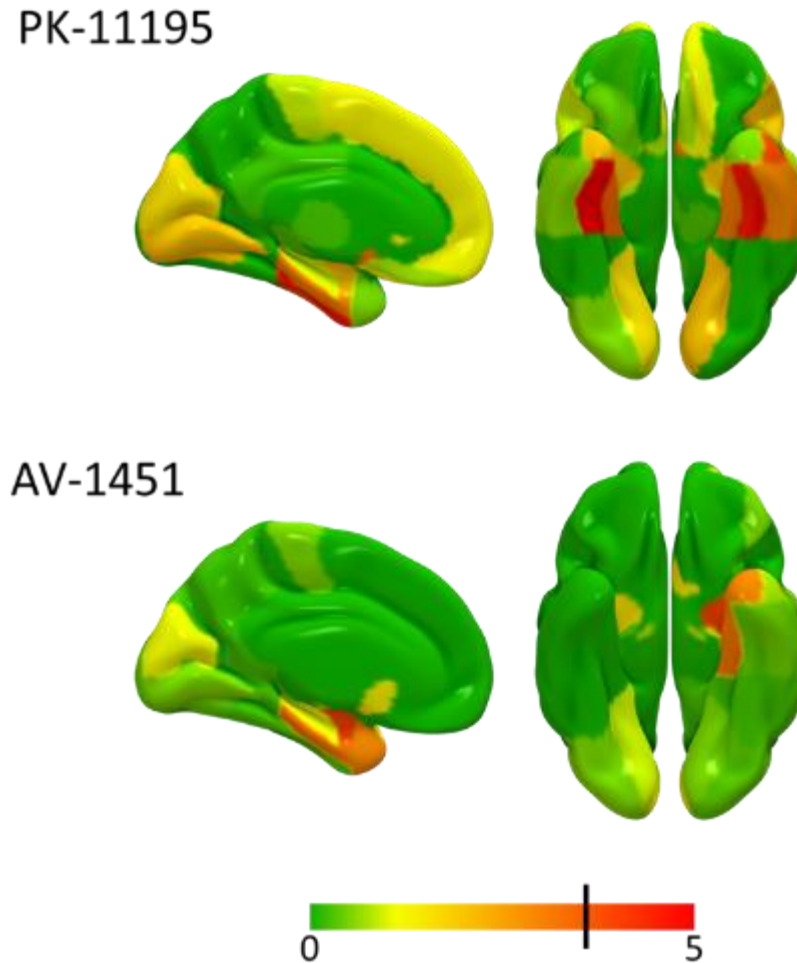


Figure 6.2: Un-thresholded maps of t-scores for the MAPT carrier against controls for PK-11195 and AV-1495. Regions coloured red (to the right of the black line on the colour scale) survived FDR correction within each modality.

The brain regions with elevated [^{11}C]PK-11195 BP_{ND} that survived correction for false discovery rate (FDR) in the MAPT carrier relative to controls were the left lateral anterior temporal lobe ($t(12)=3.88$, $q=0.046$), left fusiform gyrus ($t(12)=4.70$, $q=0.014$), and right fusiform gyrus ($t(12)=10.33$, $q<0.001$). The distribution of [^{11}C]PK-11195 BP_{ND} was clearly dissimilar in the MAPT carrier compared to controls (figure 6.3). Hierarchical clustering analysis based on average linkage classified the patient radio-ligand distribution as the most abnormal. Blood sampling at the time of [^{11}C]PK-11195 PET found a CRP $<4\text{mg/L}$ (the lower limit of test sensitivity).

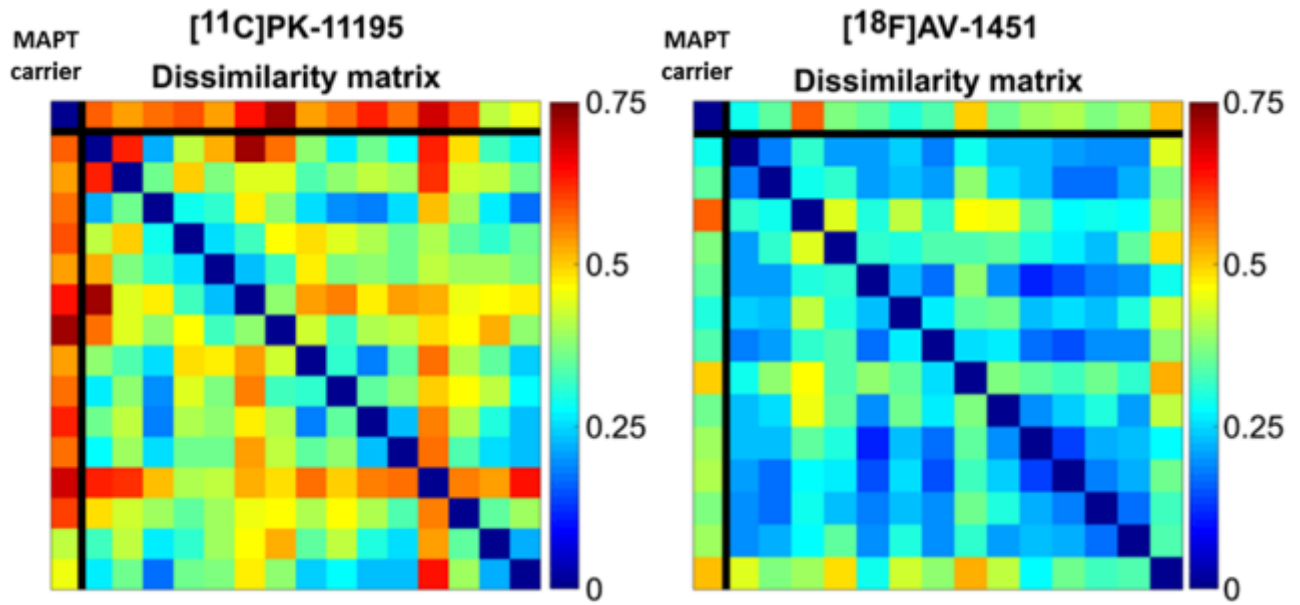


Figure 6.3: Dissimilarity matrices for across-individual whole-brain distributions of PK-11195 (left) and AV-1451 (right); for each matrix the case is represented in the first row and column, with each control providing a subsequent row and column.

No regions showed elevated $[^{18}\text{F}]\text{AV-1451}$ BP_{ND} after FDR correction. The distribution of $[^{18}\text{F}]\text{AV-1451}$ BP_{ND} was only moderately dissimilar for the MAPT carrier compared to controls (figure 6.3). Hierarchical clustering analysis based on average linkage classified the patient distribution as the third most abnormal. Atrophy in the right amygdala was the only structural change that survived FDR correction ($t(10)=5.26$, $q=0.031$). Figure 6.4 shows BP_{ND} for $[^{18}\text{F}]\text{AV-1451}$ and $[^{11}\text{C}]\text{PK-11195}$ in the control group, MAPT carrier and a previously published case with established MAPT behavioural variant FTD (Bevan-Jones et al., 2016).

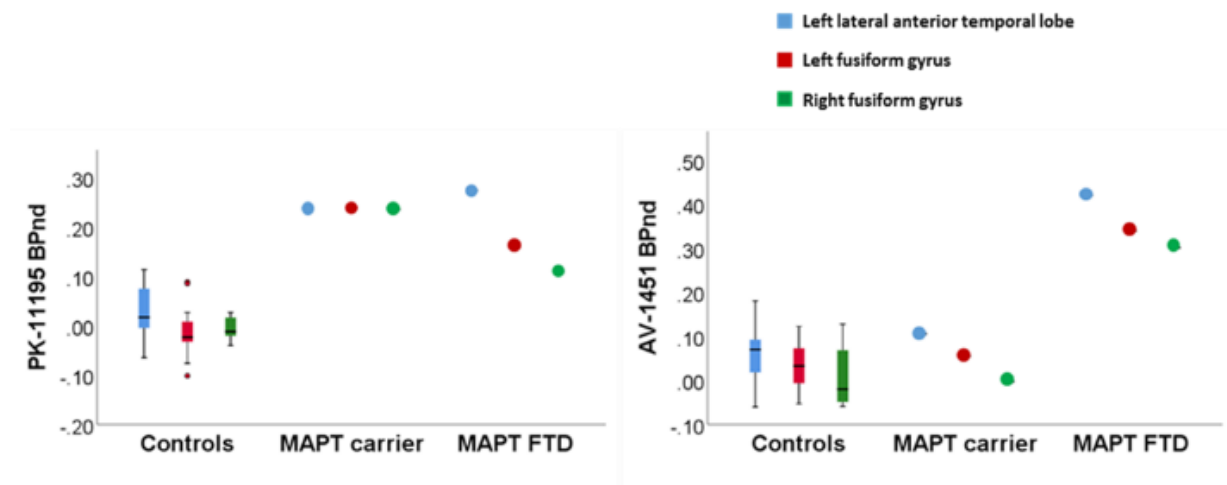


Figure 6.4: PK-11195 (left) and AV-1495 (right) binding potential for the MAPT carrier in regions with elevated PK-11195, in comparison to AV-1495 binding potential in the control groups and in a more advanced MAPT FTD case previously described in chapter 3.

Discussion

The primary finding is that the pre-symptomatic phase of MAPT mutation carriage is associated with temporal lobe neuroinflammation in the absence of significant binding of the “tau” PET ligand [^{18}F]AV-1451 (figure 6.2), even though this ligand has been shown to bind in symptomatic cases with the same mutation (Bevan-Jones et al., 2016). The multivariate, non-parametric dissimilarity matrices confirmed this by showing that the pre-symptomatic carrier was clearly discriminated from controls by her [^{11}C]PK-11195 binding distribution, but not her [^{18}F]AV-1451 binding distribution (figure 6.3).

Of note, there was only marginal grey matter atrophy (in a single, small region, the amygdala) in the pre-symptomatic MAPT mutation carrier. Together, these results suggests limited accumulation of the tau aggregates to which AV1451 binds, and minimal neuronal loss, despite the presence of neuroinflammation.

The relatively small degree and distribution of [^{18}F]AV-1451 binding, indicative of tau burden, also confirms that the proband was in the early phase of a potentially long natural history of neurodegeneration associated with a MAPT mutation (Rohrer et al., 2015). Overall these findings suggest that microglial activation precedes clinical symptom onset and significant structural changes in this hereditary tauopathy, constituting an early feature rather than a late consequence of neurodegeneration. The hypothesis that microglial activation precedes clinical symptom onset has been previously suggested (Miyoshi et al., 2010) and is supported by the comparison with an individual with symptomatic FTD due to the same 10+16 MAPT mutation (figure 6.4). The pre-symptomatic carrier demonstrated a similar level of elevation in [^{11}C]PK-11195 binding compared to this symptomatic individual, but did not demonstrate the same elevation of [^{18}F]AV-1451 binding.

There are two obvious potential interpretations of this phenomenon. Firstly, it is possible that microglial activation promotes abnormal tau aggregation. Alternatively, microglial activation might be induced prior to the presence of tau aggregates, by oligomeric tau to which [¹⁸F]AV-1451 has lower affinity. Therefore, while the data suggest an early role for inflammation in MAPT-related FTD, they do not settle the debate about whether microglial activation promotes tau aggregation, whether it is a reactive or even protective process. This question could be directly assessed in future work by mediation analysis in longitudinally examined cohorts of genetic carriers, such as that afforded by the Genetic Frontotemporal dementia Initiative (GENFI) (Rohrer et al., 2015).

This study has the limitations of a single case report, even when compared to a larger control group. However, it illustrates the potential utility of multimodal imaging in pre-symptomatic stages of FTD-related diseases to investigate the early pathophysiology of neuropathological subtypes. Whilst the MAPT carrier is pre-symptomatic, she is approaching the age at which she would be expected to manifest symptoms, and older than the age at which her sibling clinically manifested the disease. She already demonstrates the abnormalities on structural imaging and neuropsychological testing (table 1) that have been described 5-10 years before symptom onset in genetic FTL (Rohrer et al., 2015). However, her disease process is at a relatively early stage and without the clinical features necessary to meet diagnostic criteria for FTD, allowing us to be clear that abnormal microglial activation is not a feature of only late disease. One must also consider the potential for differences in the signal-to-noise of the two PET methods and MRI volumetry. Differences in data variance in the two methods would lead to differences in statistical maps, even for equivalent magnitudes of disease effects on inflammation, tau and atrophy. Against this however are the findings from the representational similarity analysis, using measures based on BP_{ND} distribution not absolute binding values. There are also limitations related to [¹⁸F]AV-1451. Unlike in Alzheimer's disease, binding in non-Alzheimer's neurodegeneration is less understood. Concerns regarding specificity relate to the demonstration of 'off-target' binding in the basal ganglia of healthy individuals (Marquie et al., 2015) and increased binding in FTD cases likely to have TDP-43 pathology (Bevan-Jones et al., 2018; Bevan-Jones et al., 2017; Makaretz et al., 2017). Binding to monoamine oxidase has also been described and is of potential relevance as isoforms may be present in neurons or in reactive astrocytes (Barrio, 2018; Vermeiren et al., 2015). However, [¹⁸F]AV-1451 is sensitive to the level and distribution of neuropathology in FTD due to the same MAPT mutation as in this case, demonstrated in chapter 3 (Bevan-Jones et al., 2016; Smith, Puschmann, et al., 2016). Finally, given the disparity in age between the case and the healthy control group, the potential confound of age warrants discussion. Both

[¹¹C]PK-11195 and [¹⁸F]AV-1451 binding are potentially influenced by age with previous studies showing increased binding with increasing age in healthy individuals (Scholl et al., 2016; Schuitemaker et al., 2012). The presence of increased binding with age would make it more difficult to find differences between a younger case and an older healthy control group rather than *vice versa*, and therefore would not be an explanation of the group differences in [¹¹C]PK-11195 binding found here. This could provide a plausible explanation for the null result found in the [¹⁸F]AV-1451 data. However, in the control groups in the dataset no significant effect of age on the binding potential of either ligand at a regional or global level could be found, suggesting that such effects, if present, are likely to be small.

Overall, the ability of PET tracers to detect pathophysiological changes upstream of neuronal loss demonstrates promise for future research in larger cohorts. Using these techniques in pre-symptomatic mutation carriers may yield insights into the pathophysiology of distinct neuropathological subtypes which, as well as leading to advances in the treatment of genetic forms of FTD, may elucidate the pathogenesis of sporadic FTD and other neurodegenerative tauopathies. Similar studies using larger patient cohorts and longitudinal assessment of the role of neuroinflammation in early-stage neurodegeneration will improve understanding of mechanisms of disease with a view to early targeted intervention.

Chapter 7: Neuroinflammation and protein aggregation across the FTD spectrum

Preface

The content of this chapter is substantially similar to a manuscript which is currently submitted and under review (Bevan-Jones et al. 'Neuroinflammation and protein aggregation co-localize across the frontotemporal dementia spectrum. bioRxiv. 2019 Jan 1:525642.'). The neuropsychological assessment was performed by myself and by Robert Arnold. Pre-processing of the PET data was performed by Dr Timothy Fryer and Dr Young Hong. Analysis and interpretation was done by myself supported by Simon Jones and Dr Thomas Cope. The text was written by myself and I am grateful for the input from my co-authors.

Summary

The clinical syndromes of frontotemporal dementia are clinically and neuropathologically heterogeneous, but processes such as neuroinflammation may be common across the disease spectrum. In this chapter I examine how neuroinflammation relates to the aggregation of tau and TDP-43 in frontotemporal dementia, and to the heterogeneity of clinical disease. I suggest that [^{18}F]AV-1451 may be a surrogate marker of non- β -amyloid protein aggregation. I assessed 31 patients with frontotemporal dementia (10 with behavioural variant frontotemporal dementia, 11 with the semantic variant of primary progressive aphasia and 10 with the non-fluent variant of primary progressive aphasia), 28 of whom underwent both [^{18}F]AV-1451 and [^{11}C]PK-11195 PET, and matched controls (14 for [^{18}F]AV-1451 and 15 for [^{11}C]PK-11195). Using univariate region-of-interest analyses, and multivariate analysis of the distribution of binding that explicitly control for individual differences in ligand affinity for TDP-43 and different tau isoforms, I found differences between patients and controls in frontotemporal regions for both neuroinflammation and protein aggregation, and a strong positive correlation between these two processes in all disease groups. Despite this regional co-localisation, the multivariate distribution of [^{11}C]PK-11195 binding related better to clinical heterogeneity than did the distribution of [^{18}F]AV-1451: distinct spatial modes of neuroinflammation were associated with different frontotemporal dementia syndromes and supported accurate group classification of participants. These *in vivo* findings indicate a close association between neuroinflammation and protein aggregation in frontotemporal dementia. The inflammatory component may be important in shaping the clinical and neuropathological patterns of the diverse clinical syndromes of frontotemporal dementia.

Introduction

Frontotemporal dementia (FTD) encompasses a clinically and pathologically heterogeneous group of neurodegenerative conditions, including the behavioural variant (bvFTD) (Rascovsky et al., 2011), non-fluent variant primary progressive aphasia (nfvPPA) and semantic variant primary progressive aphasia (svPPA) (Gorno-Tempini et al., 2011). In recent years, attention has focused on understanding the pathogenic role of protein misfolding and aggregation, which is a cardinal feature of the *post mortem* diagnostic criteria for frontotemporal lobar degeneration (MacKenzie et al., 2010). However, there are several different pathological proteins and aggregation morphologies in FTD, with generally weak correlations between clinical syndrome and the type of pathological protein (Seelaar, Rohrer, Pijnenburg, Fox, & van Swieten, 2011) (with the exception of svPPA, which is strongly associated with TDP-43 type C neuropathology (Spinelli et al., 2017)). However, other neuropathological processes may be present in common across these diverse clinical syndromes and present potential therapeutic targets. In particular, there is converging evidence for the role for neuroinflammation in neurodegenerative dementias, including FTD, from genetic associations (Broce et al., 2018; Guerreiro et al., 2013; Rayaprolu et al., 2013), cerebrospinal fluid (Sjogren, Folkesson, Blennow, & Tarkowski, 2004; Woollacott et al., 2018), epidemiology (Miller et al., 2013, 2016), *post mortem* tissue (Lant et al., 2014; Venneti, Wang, Nguyen, & Wiley, 2008) and animal models (Bhaskar et al., 2010; Yin et al., 2010; Yoshiyama et al., 2007). Both the intensity of neuroinflammation, and its distribution across the brain, may be relevant determinants of the clinical syndrome.

Positron emission tomography (PET) allows the topographic quantification of specific molecules using radioligands. In this study, I measured neuroinflammation and protein aggregation *in vivo* in patients with bvFTD, svPPA and nfvPPA, to answer key questions regarding the relationship of these pathophysiological processes. [¹¹C]PK-11195, which binds to the translocator protein (TSPO) that is expressed on the outer mitochondrial membrane of activated microglia, is a robust and sensitive marker of microglial activation with an established role as a proxy for neuroinflammation in neurodegenerative diseases (Stefaniak & O'Brien, 2015). [¹⁸F]AV-1451 was originally developed to bind to paired helical filament tau in Alzheimer's disease (Chien, Bahri, Szardenings, Walsh, & Mu, 2013; Xia et al., 2013; Zhang et al., 2012), and has been extensively used in Alzheimer's and non-Alzheimer's diseases. Elevated *in vivo* binding is seen in tauopathies characterised by straight filaments (Bevan-Jones et al., 2016; Jones et al., 2018; Passamonti et al., 2017; Smith et al., 2017), albeit with generally lower binding affinity than in Alzheimer's disease, and also in TDP-43 related disease (Bevan-Jones et al., 2018; Bevan-Jones et al., 2017; Makaretz et al.,

2017). It also has low affinity for β -amyloid and α -synuclein (Xia et al., 2013). Therefore, although the molecular interpretation of increased binding is incompletely understood (Marquié et al., 2015; Sander et al., 2016), this elevated *in vivo* binding suggests [^{18}F]AV-1451 may represent a proxy index of aggregated non- β -amyloid pathological proteins across the FTD spectrum. Given the evidence for differences in affinity of [^{18}F]AV-1451 for different tau and TDP-43 conformational targets, our analysis strategy concentrates on the relative topographical distribution of binding across regions within each individual, rather than the simple magnitude of binding. In this way I explicitly control for difference in binding affinity between syndromes and protein strains within each syndrome.

I test the hypotheses that, in FTD, neuroinflammation and protein aggregation are both increased in frontotemporal regions compared to controls, and that neuroinflammation and protein aggregation co-localise in each FTD syndrome, consistent with the syndrome-specific neuropathological distributions (e.g., co-localization of neuroinflammation and protein aggregation in the temporal pole of patients with svPPA). I use data driven approaches to elucidate the spatial modes of neuroinflammation associated with FTD, and machine learning based on multi-dimensional scaling of distributional dissimilarities, to investigate whether the cortical distribution of neuroinflammation and protein aggregation can accurately discriminate diagnostic groups thereby illustrating their mechanistic importance.

Methods

As part of the NIMROD study (Bevan-Jones et al., 2017), 31 patients (10 with bvFTD, 11 with svPPA and 10 with nvPPA) underwent PET scanning with [^{18}F]-AV1451. 28 of the 31 (9 with bvFTD, 9 with svPPA and 10 with nvPPA) also underwent a PET scan with [^{11}C]PK-11195. The order of scans was randomised. Fourteen healthy control participants underwent [^{18}F]AV-1451 PET and, to minimise radiation exposure in healthy individuals, a different group of 15 healthy participants underwent [^{11}C]PK-11195 PET scanning. Genetic and β -amyloid status (by PET or cerebrospinal fluid biomarkers) for patients were tested if clinically indicated.

PET with [^{18}F]AV-1451 and [^{11}C]PK-11195 was performed on a GE Discovery 690 PET/CT (GE Healthcare) with a low dose CT for attenuation correction or on a GE Advance PET scanner (GE Healthcare) with a 15-min $^{68}\text{Ge}/^{68}\text{Ga}$ transmission scan for attenuation correction. The PET scan itself used dynamic imaging for 90 ([^{18}F]AV-1451) and 75 ([^{11}C]PK-11195) minutes respectively. All radioligands were prepared at the Wolfson Brain Imaging Centre (WBIC), University of Cambridge, with high radiochemical purity (>95%). Each subject underwent contemporaneous 3T magnetic resonance imaging (MRI) using a

Siemens Magnetom Skyra, Verio or Tim Trio (www.medical.siemens.com). A high-resolution T1 weighted sequence was acquired (176 slices of 1.0 mm thickness, TE= 2.98 ms, TR = 2300 ms, flip angle =9°, acquisition matrix 256x240; voxel size = 1x1x1 mm³) and used for tissue segmentation (grey and white matter along with CSF), and for non-rigid registration of standard space regions of interest. For both ligands, non-displaceable binding potential (BP_{ND}) was calculated in 83 regions of interest, defined by a Hammers atlas modified to include the midbrain and the dentate nucleus of the cerebellum, by kinetic modelling using a simplified reference tissue model, with cerebellar grey matter as reference region for [¹⁸F]AV-1451 (Passamonti et al., 2017) and supervised cluster analysis used to define the [¹¹C]PK-11195 reference region (Yaqub et al., 2012). Prior to kinetic modelling all region of interest data were corrected for cerebrospinal fluid contamination of the region (i.e. partial volume corrected) through division by the mean region grey plus white matter fraction, determined using tissue probability maps smoothed to PET spatial resolution.

Four data analysis approaches were used, each designed to answer a different focused question and to explicitly control for expected between-subject and between-region differences in ligand affinity.

As a first-stage data exploration of between-group differences, a repeated-measures ANOVA was performed across the 83 regions, including age as a covariate and Greenhouse-Geisser penalisation of degrees of freedom to correct for non-sphericity. *Post hoc* t-tests were then performed between each group, corrected for false discovery rate (FDR) over regions.

Second, to examine the relationship between neuroinflammation and protein aggregation in each disease group, a correlation between the regional BP_{ND} of each ligand was performed. PET scanning with any ligand characteristically results in a general pattern of lower BP_{ND} in brain regions such as temporal lobe and higher BP_{ND} in deep brain nuclei. I was concerned that such non-specific effects might drive apparent correlations, and weak correlations were observed between our cohorts of controls for each ligand (figure 7.1). To control for this, I examined the between-ligand correlation within each disease group both with and without subtraction of the control mean BP_{ND} for each of the 83 regions of interest.

Third, to elucidate the topographical patterns of inflammation and protein aggregation in FTD, I entered the BP_{ND} of each ligand in each of the 83 regions of interest into a principal component analysis. Components were retained by Cattell's criterion (i.e. to the elbow of the Scree plot) and then tested for group differences across diagnosis in a repeated measures ANOVA. *Post hoc* t-tests examined group differences in the expression of each

topographical pattern. These first three analyses were performed in SPSS Statistics version 25 (IBM).

Finally, I undertook an analysis of the relative distribution of ligand binding potential for each ligand for every individual. This used previously published non-parametric methods (Bevan-Jones et al., 2016), that were explicitly designed to control for between-subject differences in the scaling of each ligand, such as might result from differences in the affinity of [^{18}F]AV-1451 for different conformations of tau or TDP-43, as well as spatial dependence between adjacent regions in PET data due to signal spread. These methods can be conceptualised as analogous to multi-voxel pattern analysis techniques for fMRI (Kriegeskorte, Mur, & Bandettini, 2008), but rather than attempting to classify observed stimuli within an individual on the basis of their representational similarity, here I am attempting to classify individuals on the basis of the similarity of relative ligand BP_{ND} distributions within their brain, blinded to overall differences in binding affinity. To do this, for each ligand and every individual separately, the parcellated data were converted to 83-element linear vectors. For each ligand separately, the resultant vectors were non-parametrically correlated (Spearman's rho) pairwise between individuals, resulting in two matrices that represented the similarity of each individual's scan to each other individual for that ligand. The inverse of these matrices (i.e. the between-individual dissimilarities) were used to calculate a two-dimensional scaling for each disease sub-group pair, using the squared metric stress distance criterion of the 'mdscale' function in Matlab R2017b (Mathworks). The resulting locations in two-dimensional space formed the inputs to a ten-fold cross-validated linear support vector machine (CV-SVM) for between-group classification based on each ligand separately. Statistical significance of the classification was assessed by comparison of the loss function of the CV-SVM against a null distribution of loss functions created by 1000 repetitions of the same procedure for identical data but shuffled group assignment labels. For those individuals who underwent scanning with both ligands, the CV-SVM process was repeated on multi-modal, four-dimensional scaling.

Results

Summary demographics are outlined in table 7.1, and individual neuropsychological test scores, motor features, genetic and CSF status for each participant are provided in table 7.2. Within the bvFTD group, two patients were positive for pathogenic mutations in the microtubule associated protein tau (MAPT) and three for expansions in C9 open reading frame 72 (C9orf72). One of the nvPPA group had a mutation in progranulin (GRN). CSF or PET β -amyloid status was assessed in 6 participants (4 with svPPA, and 2 with nvPPA), all of whom were negative.

Group	Number	M:F	Mean age	Education years	ACE-R /100	MMSE /30	FAB /18	FTDRS Logit	FTDRS Percent /100
nvPPA	10	3:7	71	12	79	27	11	1.92	71.7
svPPA	11	9:2	68	14	63	25	12	0.74	52.8
bvFTD	10	5:5	60	13	57	22	8	-2.46	17.4
Tau controls	14	7:7	67	16	95	29	--	--	--
PK controls	15	7:8	69	14	92	29	--	--	--
		b	a, d	a, b, d	a, b, c, d	a, d			

Table 7.1: Summary demographics and neuropsychometry: a=F test significant $p<0.05$ across all groups, b= $p<0.05$ significant pairwise comparison nvPPA vs combined control group, c= $p<0.05$ significant pairwise comparison svPPA vs combined control group, d= $p<0.05$ significant pairwise comparison bvFTD vs combined control group. Pairwise comparisons are by t-test for each demographic except sex comparison by Chi-squared.

Case	Diagnosis	Gene/ β -amyloid status	Sex	Entry age	Education years	ACE-R /100	MMSE /30	FAB /18	FTDRS Logit score	Motor features
1	nfvPPA	A β -ve (CSF)	M	55	14	93	29	15	3.35	-
2	nfvPPA	-	F	67	16	88	28	13	2.19	-
3	nfvPPA	A β -ve (CSF)	F	62	11	90	27	15	1.92	+
4	nfvPPA	-	F	84	11	85	30	11	5.39	+
5	nfvPPA	-	F	81	10	78	28	15	0.16	-
6	nfvPPA	-	F	74	10	40	16	7	-0.8	-
7	nfvPPA	GRN	M	66	10	76	22	9	-0.2	-
8	nfvPPA	-	F	77	11	86	30	13	0.34	+
9	nfvPPA	-	M	74	11	87	30	10	1.47	+
10	nfvPPA	-	F	70	11	71	25	6	5.39	-
11	svPPA	-	M	77	16	45	22	11	-1.27	-
12	svPPA	-	M	69	16	77	28	11	-1.54	-
13	svPPA	A β -ve (CSF)	M	61	15	79	30	16	-0.4	-
14	svPPA	A β -ve (PiB)	F	65	18	72	27	16	-	-
15	svPPA	A β -ve (CSF)	M	67	17	71	27	17	2.49	-
16	svPPA	A β -ve (CSF)	M	65	13	68	27	13	5.39	-
17	svPPA	-	M	72	13	63	25	12	1.26	-
18	svPPA	-	F	63	10	59	26	14	0.7	-
19	svPPA	-	M	69	18	85	30	14	2.19	-
20	svPPA	-	M	63	10	61	27	8	-0.8	-
21	svPPA	-	M	72	9	9	3	0	-0.59	-
22	bvFTD	-	F	63	12	79	29	11	-3.09	-
23	bvFTD	-	M	61	11	47	15	5	-2.18	-
24	bvFTD	MAPT	F	50	16	43	21	9	-3.8	-
25	bvFTD	-	M	75	16	68	21	6	-0.4	+
26	bvFTD	MAPT	F	70	16	38	14	7	-3.09	-
27	bvFTD	-	F	67	11	71	28	8	-0.8	-
28	bvFTD	-	M	51	14	81	29	11	-2.58	-
29	bvFTD	C9orf72	M	56	10	53	25	6	-1.03	+
30	bvFTD	C9orf72	F	51	10	41	16	7	-3.8	-
31	bvFTD	C9orf72	M	58	9	46	17	5	-3.8	-

Table 7.2: Demographics, neuropsychological testing, genetic/ β -amyloid status and motor phenotype for each disease participant. A β -ve: negative tests for β -amyloid by cerebrospinal fluid biomarkers (CSF) or Pittsburgh compound B PET scan. MAPT: microtubule associated protein tau.

The regional correlation between binding potentials of the two ligands in control participants is shown in figure 7.1.

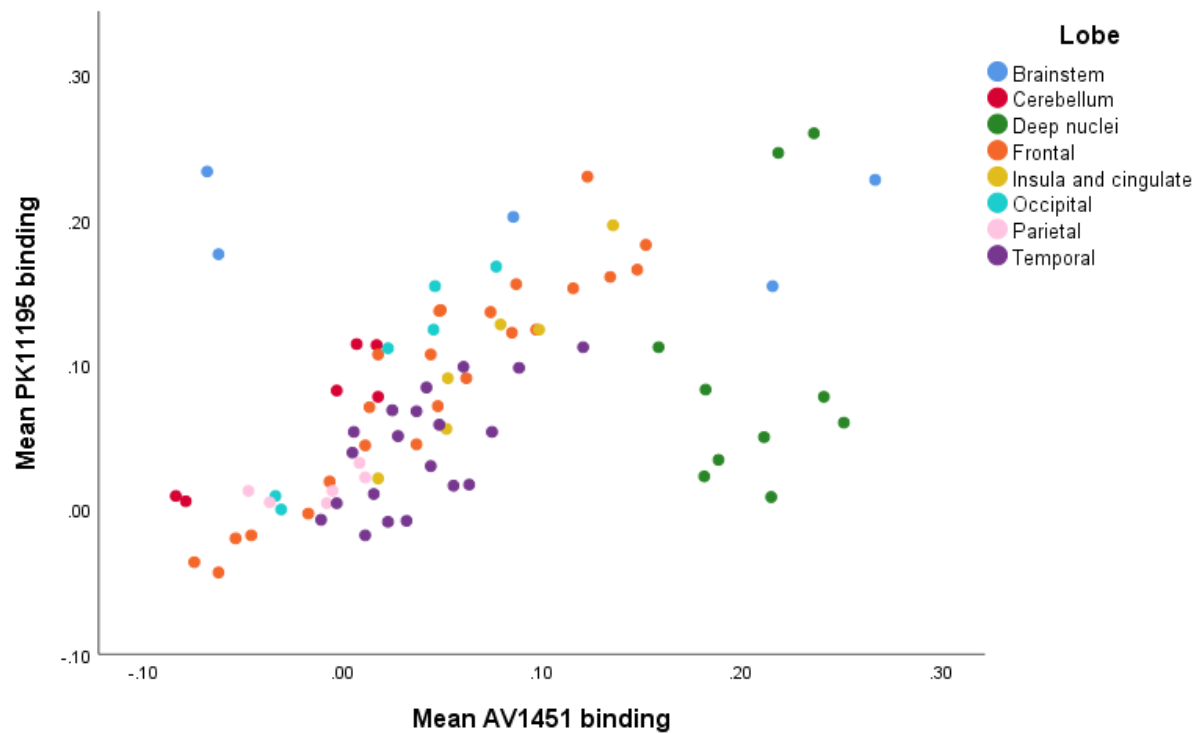


Figure 7.1: Plot of control participants of regional mean PK11195 binding against mean regional AV1451 binding

Group comparisons of frontotemporal dementia with controls

The repeated-measures ANOVA of regional [^{11}C]PK-11195 binding across the FTD groups and controls demonstrated a significant interaction between region and diagnosis ($F(39.5, 500.6)=9.2, p<0.0001$). T-maps from the post-hoc pairwise comparisons between the control group and each disease group are shown in figure 7.2. After correction for FDR, regions with significantly elevated binding were: in the bvFTD group; bilateral superior frontal gyri and putamen, right nucleus accumbens, left posterior orbital gyrus, inferior frontal gyrus and middle frontal gyrus. In the svPPA group; left insula, middle and inferior temporal gyri, right superior parietal gyrus, middle and inferior temporal gyrus, bilateral postcentral gyri, superior temporal gyrus, parahippocampal and ambient gyri, amygdala, inferior lateral anterior temporal lobe, medulla, nuclei accumbens, medial anterior temporal lobe, fusiform gyri. In the nvPPA group no differences survived FDR correction but the peak t-score was in left inferior frontal gyrus ($t(23)=2.17$, uncorrected $p=0.04$), which would be expected *a priori* to be the disease epicentre (Rogalski, Cobia, Harrison, Wieneke, Thompson, et al., 2011).

The repeated measures ANOVA of regional [^{18}F]AV-1451 binding across the FTD groups and controls showed a significant interaction between region and diagnosis ($F(33.4,$

445.1)=10, $p<0.0001$). T-maps from the post-hoc pairwise comparisons between the control group and each disease group are shown in figure 7.2. After correction for FDR, significantly elevated binding was seen in svPPA, in the following regions; left amygdala, fusiform, medial anterior temporal lobe, middle and inferior temporal gyri and superior temporal gyrus, bilateral inferolateral anterior temporal lobes.

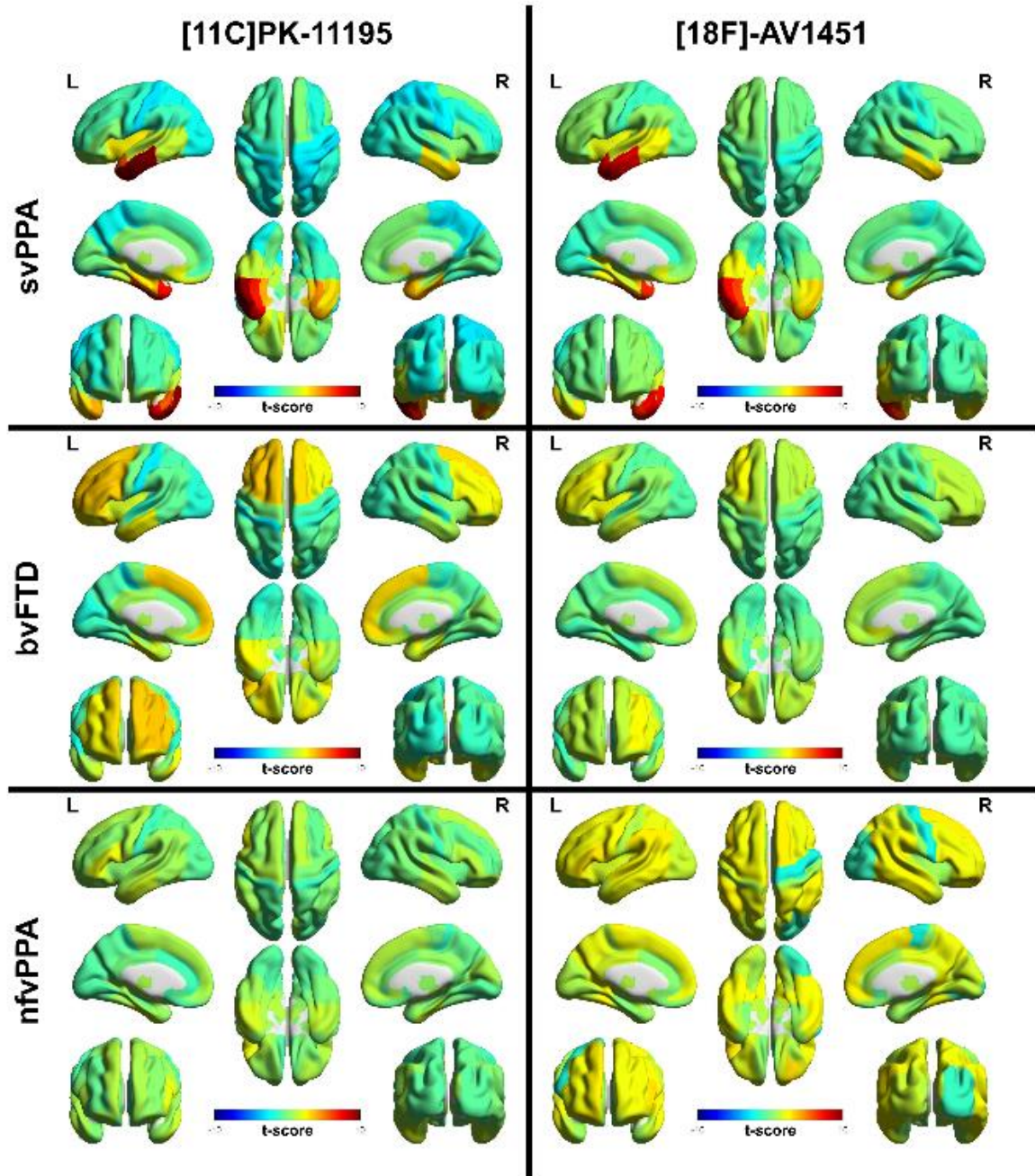


Figure 7.2: Unthresholded regional T-scores for each disease group compared to the control group for [11C]PK-11195 BPND in the left column and [18F]AV-1451 BPND in the right column.

Correlation of [^{11}C]PK-11195 with [^{18}F]AV-1451 in frontotemporal dementia

Regional control adjusted group mean [^{11}C]-PK11195 BP_{ND} was strongly correlated with regional group mean [^{18}F]-AV1451 BP_{ND} in each group both before and after the subtraction of the control group values in every region: svPPA ($r(81) = 0.727$, $p < 0.0001$ before, $r(81) = 0.883$, $p < 0.0001$ after), bvFTD ($r(81) = 0.582$, $p < 0.0001$ before, $r(81) = 0.499$, $p < 0.0001$ after), and nvfPPA ($r(81) = 0.427$, $p < 0.0001$ before, $r(81) = 0.589$, $p < 0.0001$ after) (figure 7.3).

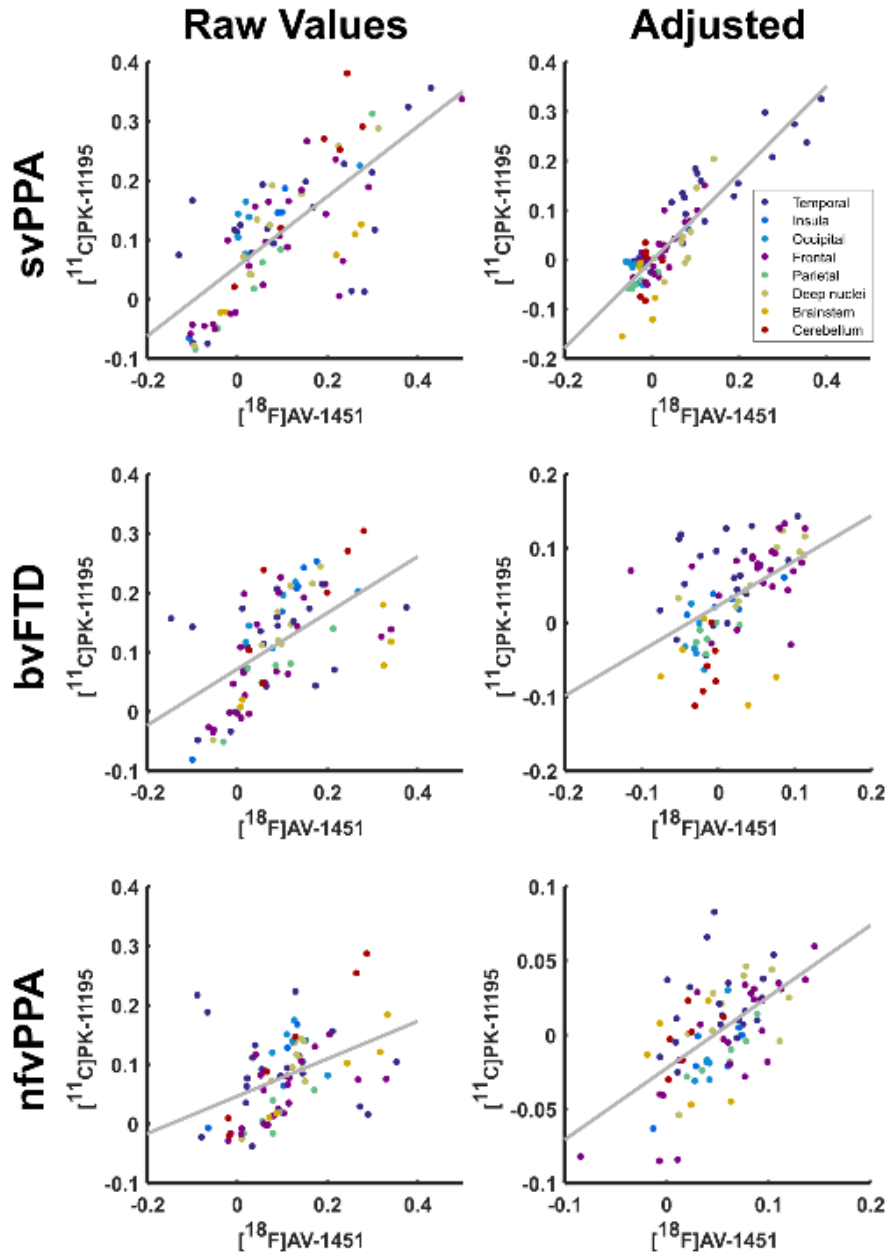


Figure 7.3: Scatter plot of the regional mean BPND for [^{11}C]PK-11195 against regional mean BPND of [^{18}F]AV-1451 by disease group.

Principal component analysis of [¹¹C]PK-11195 and [¹⁸F]AV-1451

Four principal components were detected in the [¹¹C]-PK11195 BP_{ND} data before the elbow of the scree plot, which together explained 64% of the variance in the data (figure 7.4). Component 1 reflected whole brain binding. Component 2 was strongly weighted to the bilateral anterior temporal lobes. Component 3 primarily comprised frontal binding with a right sided predominance. Component 4 was not strongly loaded onto any single region but was weighted towards motor cortex. In a repeated measures ANOVA including these 3 principal components, there was a main effect of diagnosis ($F(3, 39)=20.8$, $p<0.0001$) and a significant interaction between principal component weighting and diagnosis ($F(5.307, 68.99)=9.885$, $p<0.0001$). *Post hoc* t-tests between individual disease groups and controls showed svPPA was associated with an increase in component 2 ($t(10.2)=8.3$, $p<0.0001$), bvFTD associated with both increased component 2 ($t(9.297)=3.37$, $P=0.008$) and component 3 ($t(8.85)=3.95$, $p=0.003$) and nvPPA associated with increased component 3 ($t(23)=2.68$, $p=0.013$) (figure 7.4). Components 1 and 4 did not significantly differ between controls and any disease group.

Five principal components were detected in the [¹⁸F]AV-1451 BP_{ND} data before the elbow of the scree plot, which together accounted for 76% of the variance in the data. Component 1 again reflected global binding but less marked in the temporal poles, which were loaded onto component 2 (left) and component 4 (right). Component 3 was weighted asymmetrically towards frontal lobe binding. Component 5 was not strongly loaded onto any single region but was weighted towards bilateral superior temporal poles. In a repeated measures ANOVA including these 5 principal components, there was a main effect of diagnosis ($F(3, 41)=5.43$, $p=0.003$) and a significant interaction between principal component weighting and diagnosis ($F(11, 150.6)=3.68$, $p<0.0001$). The bvFTD group had increased weightings in component 3 ($t(22)=2.345$, $p=0.28$) and component 4 ($t(11.575)=3.284$, $p=0.007$), and svPPA had increased weightings in component 2 ($t(15.005)=6.819$, $p<0.0001$) and component 4 ($t(12.9)=2.475$, $p=0.028$) (figure 7.4). There were no significant *post hoc* differences between nvPPA and controls. Components 1 and 5 did not significantly differ between controls and any disease group.

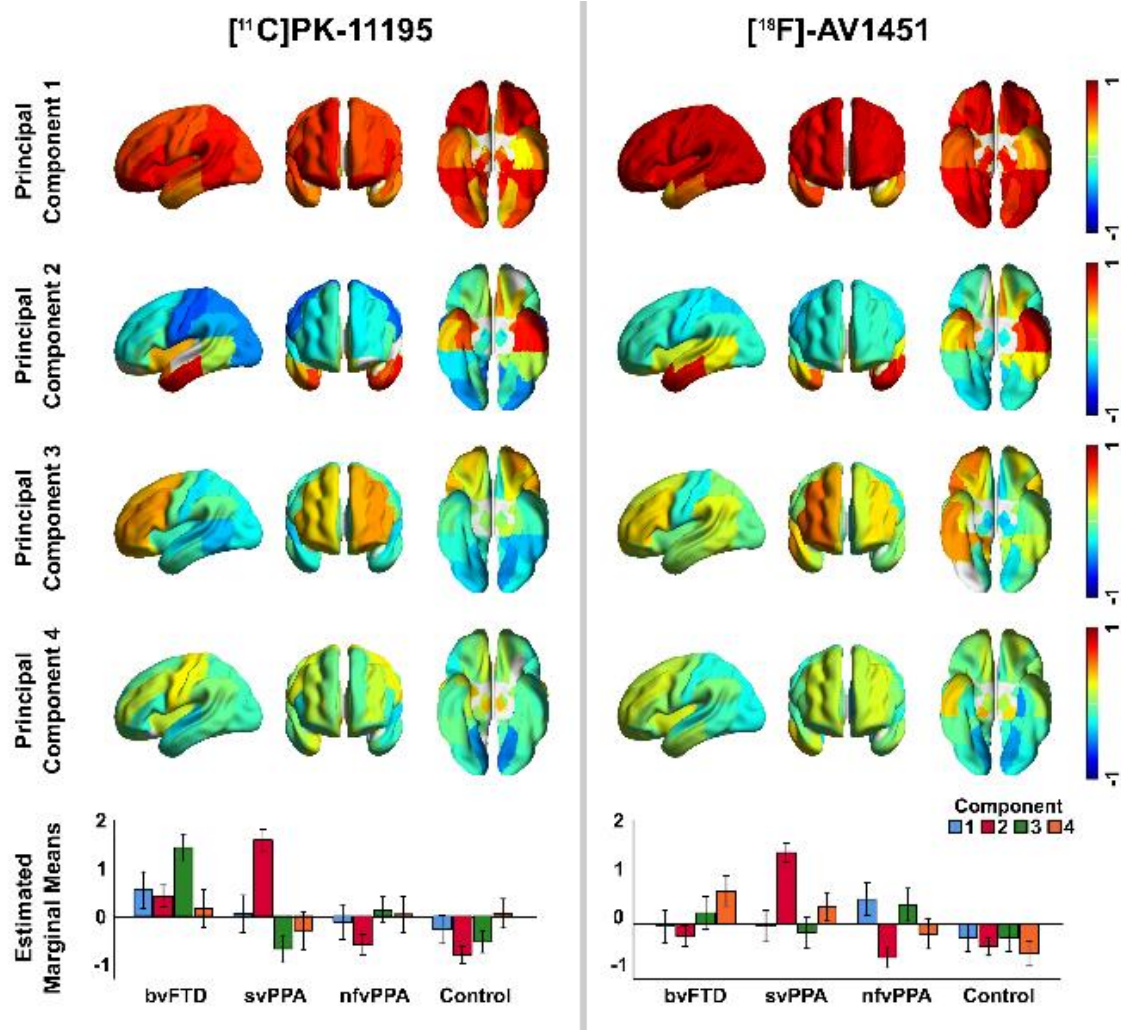


Figure 7.4: First four principal components for $[^{11}\text{C}]\text{PK-11195}$ in the left column and $[^{18}\text{F}]\text{AV-1451}$ in the right column. $[^{18}\text{F}]\text{AV-1451}$ component 5 was also retained by Cattell's criterion but was not strongly weighted to any region and did not discriminate groups so is omitted here for parsimony. The bottom row plots the estimated marginal means from the repeated measures ANOVA for each ligand, illustrating the association between principal component and diagnosis for each ligand.

Non-parametric analysis of $[^{11}\text{C}]\text{PK-11195}$ and $[^{18}\text{F}]\text{AV-1451}$ distributions

The principal component analyses suggest that a large amount of the variance between-subjects relates to whole brain PET signal. While this might reflect global differences in protein aggregation and neuroinflammation, it could also be explained by variations in radioligand affinity for different protein pathologies or other non-specific influences discussed below. I therefore performed an analysis of the relative *distribution* of PET signal for each individual scan, blinded to differences in overall signal magnitude by non-parametric rank-order statistical methods.

Multi-dimensional scaling plots of the non-parametric similarity between ligand distributions, for each sub-group pair and for all groups combined, are shown in figure 7.5. The CV-SVM classification accuracy and permutation-based statistical significance are indicated next to

each plot. Classification was significantly better than chance in all cases, except for the finding that the non-parametric distribution of [^{18}F]AV-1451 was unable to distinguish between bvFTD and nvPPA.

For those FTD participants that underwent scanning with both ligands, the classification procedure was repeated after combining the multi-dimensional scaling data such that the CV-SVM operated on four dimensions rather than two. This resulted in an improvement in the differentiation of bvFTD and svPPA compared to either ligand alone (88.9% classification accuracy, $p < 0.001$). Multimodal nvPPA vs svPPA classification accuracy matched the performance of [^{11}C]-PK11195 at 100%, $p < 0.001$, but bvFTD vs nvPPA classification performance was intermediate compared to each ligand alone, at 73.7%, $p = 0.033$.

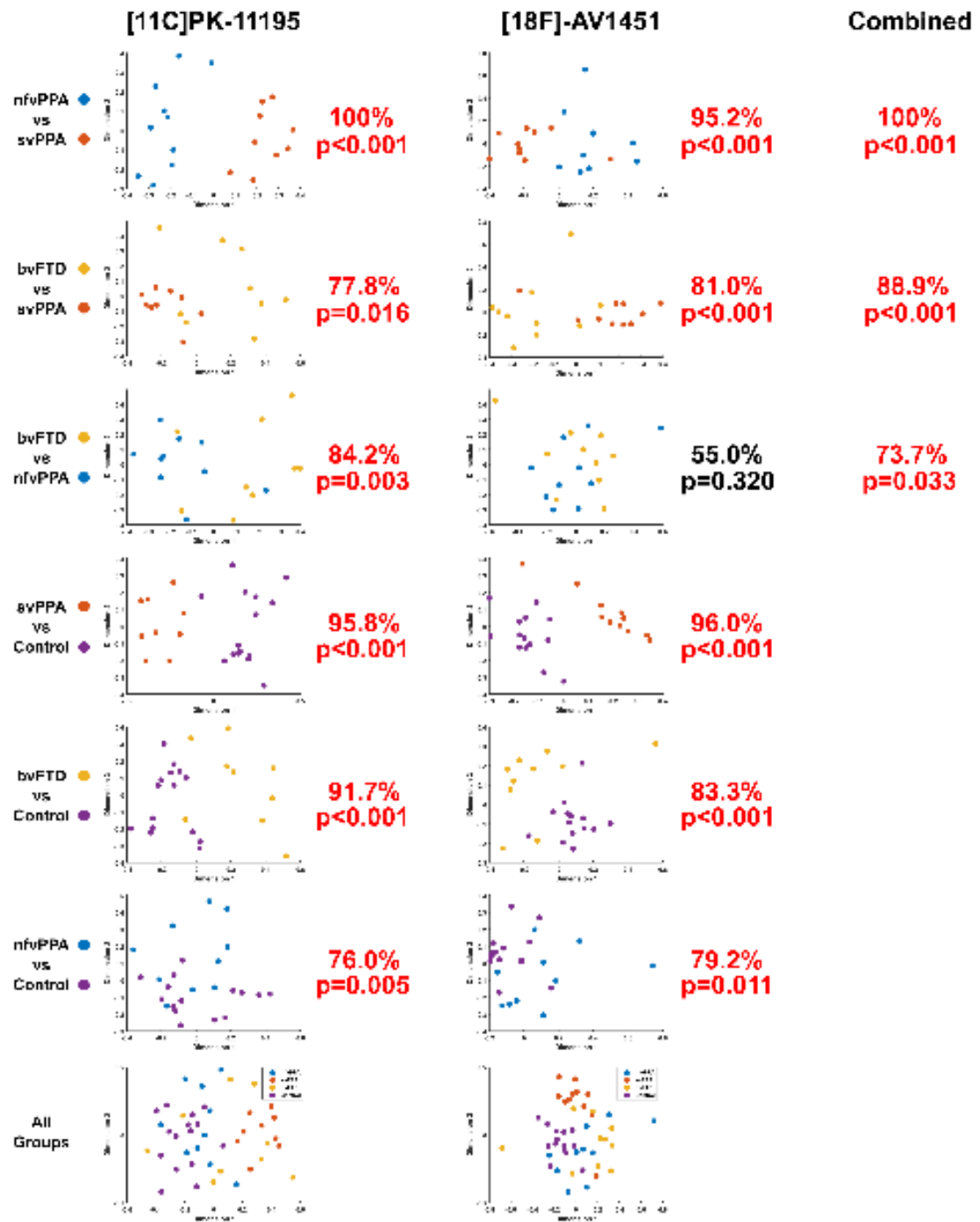


Figure 7.5: Pairwise classification accuracy for each ligand: $[^{11}\text{C}]\text{PK-11195}$ on the left, $[^{18}\text{F}]\text{AV-1451}$ in the middle, and using combined data on the right. The graphs represent a two-dimensional projection of the between-individual PET signal distribution dissimilarity according to the squared metric stress criterion. A ten-fold cross-validated support vector machine was applied to each plot, and the classification accuracy compared to a null distribution of 1000 randomisations for non-parametric significant testing. For each comparison percentage classification and p-value is stated.

Discussion

This *in vivo* study provides insights into complementary pathophysiological processes of frontotemporal dementia. Taken as a whole, the findings support an important role for neuroinflammation across the FTD spectrum, corroborating suggestions from epidemiological (Lant et al., 2014; Venneti et al., 2008), genetic (Broce et al., 2018; Guerreiro et al., 2013; Rayaprolu et al., 2013), imaging (Cagnin, Rossor, Sampson, MacKinnon, & Banati, 2004; Miyoshi et al., 2010) and animal studies (Bhaskar et al., 2010; Yin et al., 2010; Yoshiyama et al., 2007). Using regional analysis of variance, I have shown that neuroinflammation (indexed by [^{11}C]PK-11195) and protein aggregates (tau or TDP43, as indexed by [^{18}F]AV-1451) are elevated across the FTD spectrum (figure 7.2). Furthermore, neuroinflammation is highly co-localised with protein aggregation within the individual syndromes, including most strongly in svPPA, where the predominant aggregated protein inclusions are TDP-43 rather than tau (figure 7.2). Principal component analysis also revealed distinct spatial modes of neuroinflammation, with frontotemporal, temporal pole and global distributions (figure 7.4). The weighting of these regional distributions differs between groups, supporting the regional differences in the pair-wise comparisons. The spatial modes of protein aggregation (figure 7.4) similarly reflect the well characterised distributions of pathology and are likewise weighted towards specific groups. However, the distribution of protein aggregation appears to be less focal than neuroinflammation in nvPPA. To test the distinctiveness of inflammation and aggregation patterns, and to explicitly control for non-specific, between-individual differences in ligand binding affinity, I used non-parametric multi-dimensional scaling and cross-validated linear support vector machines to classify patients. I demonstrated that the distribution of neuroinflammation can accurately distinguish each of the FTD syndromes from controls and from each other (figure 7.5). Classification was often possible based on the distribution of protein aggregation, but with less accuracy. The greater discriminatory ability of neuroinflammation emphasises its potential mechanistic relevance to the pathophysiology of FTD. Despite being strongly correlated at a regional level, the two PET tracers carry some unique information across these conditions, as illustrated by the improvement in distinguishing bvFTD from svPPA when multi-modal data were available to the classifier.

The correlation between regional distributions of neuroinflammation and protein aggregation supports a close relationship between these processes in FTD, mirroring recent evidence from Alzheimer's disease that neuroinflammation is correlated with tau aggregation (Dani et al., 2018), and extending this to TDP-43 associated diseases. One interpretation of co-localised neuroinflammation and protein aggregation is that microglial activation is an early or initiating pathophysiological process, which promotes or accelerates abnormal protein

misfolding and aggregation. Whilst it was previously thought that inflammation in the brain only occurred in the context of direct infection or after breakdown of the blood brain barrier, it is now recognised that microglia play a key role in orchestrating the innate immune response of the brain. They can be activated by misfolded proteins, and mediate responses through inflammatory pathways, cytotoxicity and changes in plasticity (Nakajima & Kohsaka, 2001; Nayak, Roth, & McGavern, 2014). In neurodegenerative diseases, this state of activation may become chronic, dysfunctional, and toxic, contributing to pathogenicity (Pasqualetti, Brooks, & Edison, 2015; Serrano-Pozo, Betensky, Frosch, & Hyman, 2016).

There is evidence for inflammatory processes in FTD (Heneka, Kummer, & Latz, 2014), from genetic (Broce et al., 2018; Guerreiro et al., 2013; Rayaprolu et al., 2013), cerebrospinal fluid (Sjogren et al., 2004; Woollacott et al., 2018), epidemiology (Miller et al., 2013, 2016), *post mortem* (Lant et al., 2014; Venneti et al., 2008) and animal studies (Bhaskar et al., 2010; Yin et al., 2010; Yoshiyama et al., 2007). It is well established that an innate immune response, characterised by activated microglia, is a feature of the neuropathology of FTD (Lant et al., 2014). Furthermore, mutations leading to haplo-insufficiency of progranulin, a growth factor that has peripheral immune and central microglial regulatory functions (Petkau et al., 2010; Pickford et al., 2011; Yin et al., 2010), leads to FTD syndromes characterised by TDP-43 pathology. Expansions in C9orf72 have effects on microglial function as well as neurons (O'Rourke et al., 2016), and risk variants for FTD in TREM2 are associated with microglial activation (Giraldo et al., 2013). Neuroinflammation is an early feature of pathophysiology in mouse models of tauopathy, where inflammatory changes precede the accumulation of aggregated tau (Yoshiyama et al., 2007) and pro-inflammatory molecules increase tau hyperphosphorylation and aggregation (Bhaskar et al., 2010). *In vivo* PET studies in small samples have shown that neuroinflammation anticipates atrophy in clinically established FTD (Cagnin et al., 2004) and precedes both symptoms and the detectability of tau aggregation by PET in MAPT mutation carriers (Bevan-Jones et al., 2018; Miyoshi et al., 2010). Although neuroinflammation appears early in the pathogenesis of FTD and other neurodegenerative disorders, it remains unclear whether it is an independently initiating factor or whether it is induced by oligomeric proteins or pre-tangles.

Much of the evidence supporting the presence of inflammation in FTD comes from *ex vivo* studies. The need to improve our understanding of this process during life has led to the development of PET radioligands for this purpose, but there is some controversy over the optimum ligand for imaging activated microglia. PET ligands which target TSPO have long been the mainstay of imaging microglia. However TSPO expression patterns in microglia are

complex and the functional effects, i.e. deleterious versus protective, of different microglial phenotypes are incompletely understood (Gomez-Nicola & Perry, 2015). Furthermore, TSPO is also expressed by other cell types, notably astrocytes (McCarthy & Harden, 1981). However, in favour of the use of [^{11}C]PK-11195 is its demonstrated selectivity for activated microglia over quiescent microglia and reactive astrocytes (Banati, 2002); its relative insensitivity to common polymorphisms in TSPO compared to second generation TSPO radioligands (Stefaniak & O'Brien, 2015; Zhang, 2015), and the fact it has well established methods of non-invasive kinetic analysis (Passamonti et al., n.d.; Turkheimer et al., 2007). [^{11}C]PK-11195 has also been effectively used in studies of other neurodegenerative diseases and shown ability to reveal pathologically-related patterns of neuroinflammation (Edison et al., 2013; Passamonti et al., n.d.; Stefaniak & Brien, 2015; Varley, Brooks, & Edison, 2015). There remain some disadvantages, including relatively high non-specific binding and low brain penetration. Whilst this signal to noise has been cited as an explanation for previous negative studies using [^{11}C]PK-11195, it does not undermine positive findings such as those shown here, especially within our multi-variate analyses that explicitly control for differences in ligand penetration and affinity. A further problem lies with interpreting the meaning of increased [^{11}C]PK-11195 binding. This must include consideration of the potential contribution of reactive astrocytes expressing upregulated TSPO, but also our incomplete understanding of the functional consequences of activated microglia and reactive astrocytosis, and the potential effects of glial neuropathology on the immune/inflammatory component of pathophysiology. Whilst it seems reasonable to determine that increased [^{11}C]PK-11195 binding equates to immune activation or neuroinflammation, the functional consequences or causality of this cannot be assumed.

In contrast to [^{11}C]PK-11195, the [^{18}F]AV-1451 binding provided a less clear signal despite such aggregation being an essential feature of FTD and many other dementias. I propose that [^{18}F]AV-1451 binding is a proxy measure of aggregated non- β -amyloid protein. In Alzheimer's disease the sensitivity of *in vivo* imaging with [^{18}F]AV-1451, and its affinity for tau in neurofibrillary tangles, is well established and has contributed significantly to our understanding of its pathogenesis and progression. However, the situation in FTD is more complex due to its pathological heterogeneity and our incomplete understanding of [^{18}F]AV-1451 binding to the various morphologies of aggregated protein observed in FTLD-tau and FTLD-TDP43 pathologies. This heterogeneity is problematic and although six of our patients have genetic mutations, and six others were β -amyloid biomarker negative (tables 1&2), we cannot definitively ascertain the majority of patients' pathological type ante mortem. In this context, the molecular targets for [^{18}F]AV-1451 binding remain controversial. Supporting our

use of [^{18}F]AV-1451 as a marker of non- β -amyloid protein aggregation, previous *post mortem* work has demonstrated some binding to FTLD pathologies, albeit at a lower magnitude than that seen with Alzheimer's pathology (Lowe et al., 2016; Marquié et al., 2015; Mcmillan et al., 2016; Sander et al., 2016). This appeared to be corroborated by *in vivo* studies of patients with a straight filament 4-repeat tauopathy and clinical FTD resulting from MAPT mutations, showing binding in areas typically affected in FTD and affected at *post mortem* (Bevan-Jones et al., 2016; Smith et al., 2016), and by the elevated binding in the affected brain regions of patients with svPPA (Bevan-Jones et al., 2017; Makaretz et al., 2017) and bvFTD due to C9orf72 expansions (Bevan-Jones et al., 2018), who have TDP-43 rather than tau pathology. However, even within genetically determined FTD binding affinity varies according to different tau isoforms and strains (Jones et al., 2018) supporting varying affinity to different morphologies of tau.

Other non-tau, non-TDP-43, targets may also account for this increased binding. It is noted that this elevated [^{18}F]AV-1451 binding is seen in a distribution that closely resembles that of neuropathology, suggesting potential binding to other proteins expressed by degenerating neurons or reactive glial cells, such as isoforms of monoamine oxidase (Vermeiren et al., 2018). Monoamine oxidase subtypes are expressed by both neurons (monoamine oxidase A) and reactive astrocytes (monoamine oxidase B) (Ben Haim, Carrillo-de Sauvage, Ceyzériat, & Escartin, 2015; Fowler, Logan, Volkow, & Wang, 2005) which may contribute to the patterns of cortical binding seen. Indeed, if [^{18}F]AV-1451 binding were driven by 'off target' binding to reactive astrocytes, which are induced by activated microglia (Liddel et al., 2017), this would only provide further evidence for the importance of neuroinflammation in FTD. Further to this, upregulation of TSPO in reactive astrocytes could occur alongside that of MAO-B accounting for the regional correlation found between ligand binding. However, the pre-symptomatic dissociation of [^{11}C]PK-11195 and [^{18}F]AV-1451 binding (Bevan-Jones et al., 2018) argues strongly against such simple cross-affinity.

In the face of uncertainty about molecular targets and variations in affinity, it is important to emphasise that through our classification analysis I focus on distribution rather than quantification of binding, using a non-parametric method that is insensitive to absolute binding values and purely reflects the pattern of binding. This takes into account the potential differences in affinity of [^{18}F]AV-1451 for different protein targets. Overall, whilst it is clear that [^{18}F]AV-1451 binding does not bind specifically to tau aggregates, the distribution of binding co-localises and varies with that expected of aggregated protein in these diseases, and *post mortem* immunohistochemistry of tau. Indeed, [^{18}F]AV-1451 may provide a usefully

non-selective marker of non- β -amyloid aggregated protein, whether tau or TDP-43, allowing *in vivo* examination across the spectrum of sporadic FTD syndromes. Whilst in the complex setting of FTLT I interpret [^{18}F]AV-1451 binding as a non-specific marker of non- β -amyloid neuropathology, the biological relevance of elevated binding in non-AD neurodegenerative disease remains incompletely understood. Further work examining [^{18}F]AV-1451 binding across large *post mortem* cohorts of FTLT pathology will be required to independently validate our hypothesis.

The main limitation of this study is group size which, although larger than most previous PET studies in FTD, is still small for each individual diagnosis. The small sample size reduces the power of the study to find parametric group differences in binding, particularly given that both ligands have a degree of insensitivity to their target, as well as limiting the ability to detect associations with clinical features and severity. Characterisation of the groups is also limited in that the genotyping and β -amyloid assays were based on clinical indications and consent: I did not directly examine β -amyloid status in all individuals and whilst there is a mix of both genetic and sporadic cases I did not genotype every participant. The inability to perform pathological subtyping *in vivo* makes interpretation of results more difficult in view of the generally poor relationship between phenotype and underlying neuropathology in FTD. Consequently, I cannot use the clinical diagnostic groups alone to draw conclusions about the relationship between microglial activation and specific forms of protein aggregation. I am also limited in the inferences about the predilection for immune dysregulation in a particular neuropathological subtype, such as the relationship suggested between immune dysfunction and FTLT-TDP-43 (Miller et al., 2013, 2016), except for the cases with genetic mutations.

To conclude, I provide *in vivo* evidence for neuroinflammation in FTD, which has a close relationship with [^{18}F]AV-1451 binding, taken in this study to represent a marker of FTLT-tau or FTLT-TDP-43 neuropathology. PET measurement of inflammation provided a more accurate classification of syndromes than did protein aggregation emphasising its potential importance in shaping the clinical and neuropathological patterns of the diverse clinical syndromes of frontotemporal dementia. A causal role for neuroinflammation in neurodegeneration would inform future drug targets and potential clinical trials in frontotemporal dementia. Our findings therefore warrant further longitudinal mechanistic investigation into the role of neuroinflammation in early-stage neurodegeneration, its relationship to specific protein aggregation and to clinical progression.

Chapter 8: General Discussion and future applications

Introduction

In this chapter I summarise the findings of the original research presented in this thesis and discuss their relevance to the literature. I emphasise the role of inflammation, and how it can be understood in relation to other aspects of pathology, in particular the abundance of intracellular protein aggregates. I comment on advantages and limitations of the PET ligands, and end with reflections on potential future directions of research.

Summary of findings

I investigated both neuroinflammation and protein aggregation *in vivo* in clinically diagnosed FTD. Initially I established that the ‘tau ligand’ [¹⁸F]AV-1451 was sensitive to a straight filament 4-repeat FTLD-tauopathy resulting from a 10+16 mutation in the MAPT gene, which is biochemically and morphologically distinct from the paired helical filament balance 3-repeat/4-repeat tauopathy of Alzheimer’s disease. However, I then established that [¹⁸F]AV-1451 is not specific for tau pathology in FTD, by demonstrating increased anatomically appropriate binding in svPPA and in a case of FTD caused by an expansion in the C9orf72 non-coding region, both of which are characterised by TDP-43 pathology. On this basis I postulated that, whilst not selective for FTLD-tau pathology in the setting of FTD, [¹⁸F]AV-1451 may be a marker of aggregated non- β -amyloid protein, be it tau or TDP-43.

Using region-of-interest and data driven analyses to assess both neuroinflammation and protein aggregation across the clinical and genetic spectrum of FTD, I have shown that both processes are increased in frontal and temporal regions compared with healthy volunteers, in a topographical pattern which reflects the known distributions of neuropathology in FTD. The two processes are also co-localised, in that where there is more neuroinflammation there is more protein aggregation. Despite this co-localisation, individual classification of participants was more accurate using the whole brain distribution of neuroinflammation rather than that of protein aggregation.

In a pre-symptomatic carrier of a 10+16 MAPT mutation, I have shown increased neuroinflammation in the temporal lobe regions classically affected in this illness, in the absence of significant tau aggregation, despite the known sensitivity of [¹⁸F]AV-1451 for the tauopathy caused by this mutation. Taking all these findings together, this thesis provides evidence for the *in vivo* presence of both neuroinflammation and protein aggregation in FTD. In particular, the role of neuroinflammation is emphasised as it appears to occur early,

potentially preceding significant tau aggregation in genetic tauopathy, but also as it has patterns which both better reflect known patterns of neuropathology in FTD and which are more strongly associated with diagnostic group than those of protein aggregation. These findings are important because they provide *in vivo* support for evidence from *in vitro*, *post mortem* and animal studies, and also because they demonstrate that it is feasible and informative to investigate pathophysiological processes such as these *in vivo*.

Pathophysiology of FTD

Although FTD is clinically and pathologically heterogeneous, a clearer picture is emerging of the underlying biology of disease and in particular the role of specific processes such as aggregation of toxic protein species and neuroinflammation. In this section I discuss the *ex vivo* evidence supporting these two processes in FTD with reference to the FTD literature and also to lessons learned from the most common neurodegenerative disease, Alzheimer's disease.

Protein aggregation

Most neurodegenerative diseases are associated with accumulation of modified and misfolded proteins, for example aggregates of tau, β -amyloid, TDP-43 and α -synuclein are all neuropathological hallmarks of specific diseases. There is also comprehensive evidence implicating these modified and misfolded protein species as toxic drivers of neurodegenerative disease. This has gained support from genetic studies which showed that mutations in genes which code for proteins characteristic of particular neurodegenerative diseases, such as the amyloid precursor protein (APP) in Alzheimer's disease and MAPT in FTD, are sufficient to cause disease. The nature of the toxicity of protein species and the mechanisms of spread have been extensively investigated in animal and *in vitro* models. Much of this work has focused on tau species and mutation models, particularly in Alzheimer's disease but also in FTLD. These have established 'gain of function' and 'loss of function' as potential toxic mechanisms. It has also been noted that as well as causing neurodegeneration directly (Ballatore, Lee, & Trojanowski, 2007), tau pathology may have a toxic effect through its interaction with apolipoprotein ϵ 4 and neuroinflammation (Currais, Fischer, Maher, & Schubert, 2017; Yates, 2017), and may have functional effects via synaptic dysfunction and loss with a resultant impact on plasticity (Wang & Mandelkow, 2015). Animal and *in vitro* models investigating cellular vulnerability to and spread of tau pathology have also emphasised the hypothesis of 'prion-like' cell to cell transmission of toxic protein species as the dominant paradigm (Clavaguera, Hench, Goedert, & Tolnay, 2015; Eisele et al., 2015; Tracy & Gan, 2018; L. C. Walker & Jucker, 2015). More recently,

work in TDP-43 models has drawn parallels with this and has emphasised the importance of RNA and protein homeostasis in the neurodegenerative process of FTD (Ling et al., 2013). *Post mortem* studies have provided a structure in which to interpret the role of protein aggregation and its spread, for example in Alzheimer's disease where the cross sectional studies of Braak and Braak demonstrated that the distribution of tau pathology at *post mortem* in Alzheimer's diseases could be classified into stages (Braak & Braak, 1991). The narrative of progressive Braak stages of pathology provides a compelling story of progressive proteinopathy, and one which may be linked to progressive neuronal damage by experimental evidence and extrapolated to clinical disease. However, there has been a fundamental lack of *in vivo* evidence corroborating this by demonstrating that distribution and burden of protein pathology correlate with clinical symptoms and severity. Despite the advances in our understanding of the framework for and effects of protein aggregation, the relationship of protein aggregation and spread to disease remains incompletely understood and may differ between diseases.

Neuroinflammation

There is increasing and converging evidence for the importance of neuroinflammation across the spectrum of neurodegenerative disease (Perry et al., 2010) and the progression in our understanding of the role of neuroinflammation in neurodegeneration has followed a similar course to that of protein aggregation. Building on observational and epidemiological evidence from humans which have shown reduced incidence of neurodegenerative diseases such as Alzheimer's disease in people who have been on long term anti-inflammatory medications for other indications (McGeer & McGeer, 2007; McGeer & McGeer, 2013; Samii, Etminan, Wiens, & Jafari, 2009), the function of microglia, as the coordinators of the immune system in the central nervous system, has been studied *in vitro* and in animal models as well as genome-wide association studies. The hypotheses for immune dysfunction in FTD come from a variety of sources. These include pre-clinical evidence from cell and animal models, as well as epidemiological, genetic and pathological studies in FTD. As in other neurodegenerative diseases *post mortem* studies have demonstrated gliosis to be a core feature of the neuropathology of FTD (Lant et al., 2014). Animal models, using the main genes associated with FTD, have also repeatedly shown the presence of microglial dysfunction and neuroinflammation alongside protein aggregation. They have also illustrated the ability of immune modulation to affect this relationship and therefore modify the evolution of protein pathology. Genetic models have demonstrated that as well as having neuronal effects on protein homeostasis and aggregation, the monogenic causes of FTD also cause changes in microglial function. In patients, genome-wide association studies in FTD have implicated

several risk loci with immune and microglial functions whilst epidemiological studies have found an increased prevalence of autoimmune diseases (Broce et al., 2018; Miller et al., 2013, 2016).

Pathophysiological targets for disease modifying drugs in neurodegeneration

The focus on different aspects of pathology, such as protein aggregation and neurodegeneration, in neurodegenerative disease has been necessary to improve our understanding of the biology of disease. Improved understanding of these processes and their relationship to disease is essential as it will underpin rationales to propose and support drug development targets and also the design of clinical trials. The details of different potential approaches to these targets in disease modification is beyond the scope of this thesis however. For both protein aggregation and neuroinflammation there is a converging body of molecular, cellular, genetic and clinical evidence supporting their importance and pathogenicity. In particular animal models have improved our understanding of the relationship between processes such as protein aggregation, neuroinflammation, neuronal dysfunction and death, as well as providing evidence that modifying these processes can be beneficial (Dagher et al., 2015; Eisele et al., 2015; Gomez-Nicola et al., 2013; Kumar et al., 2016; Martínez-Muriana et al., 2016; Nash et al., 2013; Olmos-Alonso et al., 2018; Spangenberg et al., 2018). However exact mechanisms and relationships to disease phenotype and progression remain to be established. To corroborate and build upon this wealth of *post mortem*, *in vitro*, animal and epidemiological evidence we need to test these hypotheses in participants with disease during life. In order to do this we need tools which can ideally quantify and map pathophysiology *in vivo*. In short, *in vivo* evidence is the next piece of the jigsaw required to verify our understanding of pathophysiology and to underscore targets for future clinical trials. In the next section I will discuss this further with particular reference to PET.

Using PET to measure pathophysiology *in vivo*

In recent years the advent of PET ligands targeting specific protein species and allowing their visualisation, has enabled the above hypotheses about the biology of neurodegeneration to be probed *in vivo*. The first protein to be imaged *in vivo* was β -amyloid but studies correlating the amount and distribution of β -amyloid with symptoms and disease severity in Alzheimer's disease showed only weak correlations (Jagust, 2016). Coinciding with this, alternatives to, or modifications of, the 'amyloid hypothesis' began to emerge, for example with tau as the toxic protein species in Alzheimer's neurodegeneration, and this drove the development of ligands selective for tau pathology. However, it is important to note that the development of PET ligands to investigate pathophysiology has been primarily driven by the Alzheimer's

disease research field, leading to a literature of *in vivo* studies focused on Alzheimer's disease using tools developed for Alzheimer's disease. As a result in the following section I will briefly outline the current evidence in Alzheimer's disease for both 'tau PET' and 'inflammation PET' and its relevance and limitations for FTD.

The advent of 'tau ligands', particularly [^{18}F]AV-1451, has allowed *in vivo* studies in Alzheimer's disease to support the idea of tau toxicity, with evidence that quantity and distribution of paired helical filament tau are related to symptoms and severity, and that they increase longitudinally in line with disease progression, essentially recapitulating the Braak stages (Jack et al., 2018; Lowe et al., 2018; Ossenkoppele et al., 2016; Schwarz et al., 2016). Studies have also begun to address the question of how tau pathology spreads, indicating this may potentially be trans-synaptic in Alzheimer's disease (Cope et al., 2018). These findings have helped to pave the way for 'anti-tau' therapies to come to trial in Alzheimer's disease, and provided rational for anti-tau therapies in primary tauopathies such as progressive supranuclear palsy. These advancements have even led some to suggest that [^{18}F]AV-1451 should be used for diagnosis and 'staging' of Alzheimer's disease as well as providing an outcome measure in clinical trials in Alzheimer's (Hansson & Mormino, 2018; Mattsson et al., 2018; Ossenkoppele et al., 2018).

The ligands used in these initial trials were all developed to bind to Alzheimer's disease tauopathy. Whilst they are commonly thought of as 'tau specific' their specificity is actually to the conformation of the β pleated sheet conformation adopted by tau in its fibrillary form. This structure is not unique however and is shared by fibrils of other proteins such as TDP-43, α -synuclein and β -amyloid. This leads to the potential for non-specific, off-target binding and other well documented limitations which are discussed elsewhere in this thesis. Recognition of this has fuelled the development of 'second generation' ligands with the aim of optimising ligand properties to maximise affinity and selectivity of binding whilst minimising 'off target' binding (Perani et al., 2019). Some of these ligands use structures derived from first generation ligands, for example [^{18}F]RO-948 and [^{18}F]PI-2620) whilst others, such as [^{18}F]MK-6240 and [^{18}F]JNJ311, are relatively novel. Work with these ligands is still at an early stage and in small numbers of participants but thus far seems to illustrate some of the same problems, for example [^{18}F]RO-948 derived from [^{18}F]AV-1451, appears to suffer from a degree of off target binding to neuromelanin, but also show some improvements and preserved strengths, for example reduced off target binding in relevant structures such as striatum and choroid plexus seen with [^{18}F]PM-PBB3, [^{18}F]PI2620, and [^{18}F]MK6240, as well as a distribution of binding in Alzheimer's disease which recapitulates Braak stages ([^{18}F]RO-

948 and [¹⁸F]MK-6240) (Leuzy et al., 2019; Perani et al., 2019). Further research in greater numbers across the tauopathy spectrum will be necessary to characterise their strengths and limitations before they can be used effectively to enhance our understanding of these diseases. The recent description of the cryo-electron microscopic structure of tau filaments in Alzheimer's disease (Fitzpatrick et al., 2017) also opens up other avenues of identifying potential PET ligand binding sites which may be better exploited by other classes of molecules such as peptides and antibodies rather than the polyheterocyclic compounds used to date.

Similarly to 'tau PET', there is a comprehensive literature from *in vivo* PET studies of microglial activation in Alzheimer's disease. This shows evidence for early and persistent *in vivo* neuroinflammation, with a distribution of microglial activation which overlaps with that of atrophy and with that expected for neuropathology (Kreisl et al., 2017). Increased neuroinflammation is correlated with worsening cognitive and functional scores and disease severity (Kreisl et al., 2013) and has also been correlated with tau aggregation (Dani et al., 2018). Interestingly it seems that the strength of the relationship between microglial activation and tau pathology in Alzheimer's disease is increased with disease severity (Dani et al., 2018). The relationship between inflammation, progression and severity in Alzheimer's disease is complex however. A biphasic role has been postulated, where high degrees of neuroinflammation in early or prodromal disease appear to be protective (Hamelin, Lagarde, Dorothe, et al., 2018), whilst in contrast greater increases in neuroinflammation longitudinally are associated with worsening clinical and functional assessments (Hamelin, Lagarde, Potier, et al., 2018).

The importance of the Alzheimer's disease PET literature is emphasised by the scarcity of molecular PET studies in FTD where prior to the start of this thesis there were only two small studies investigating inflammation in FTD and MAPT carriers respectively (Cagnin et al., 2004; Miyoshi et al., 2010). Whilst not specifically applicable to FTD, the Alzheimer's disease literature not only generates hypotheses to be tested in FTD, but also a road map for the process of doing so. In the next section I will discuss how my findings begin this journey and what needs to be learned from this study to optimise our future approaches to PET research in FTD.

Relevance to other disorders

This thesis begins the journey of investigating *in vivo* pathophysiological processes in FTD. It is important as it provides corroborative evidence to support pre-clinical, clinical and animal model generated hypotheses for the presence of neuroinflammation, and its

relationship with protein aggregation, in the pathophysiology of FTD. They also mirror similar findings and relationships in Alzheimer's disease (Dani et al., 2018), and extend the relationship between neuroinflammation and protein aggregation to include TDP-43 associated diseases such as svPPA. The studies of [¹⁸F]AV-1451 binding in FTD have been at the forefront of the investigation of [¹⁸F]AV-1451 binding across the neurodegenerative spectrum over the last 3 years, and have contributed to the understanding of *in vivo* binding and its interpretation in non-Alzheimer's disease settings. They have helped to establish that whilst present, the sensitivity of [¹⁸F]AV-1451 to FTD-tau is less than that seen in Alzheimer's disease, and have subsequently been replicated and extended in various studies (Ali et al., 2018; Cho et al., 2018; Jones et al., 2018; Josephs et al., 2018; Smith et al., 2017). Similarly, this study was the first to publish a lack of specificity for FTD-tau cases, by demonstrating binding in FTD-TDP43 cases, a finding since validated by others (Josephs et al., 2018; Makaretz et al., 2017).

Through these findings this thesis has begun to address some of the fundamental research requirements in the FTD field, with relevance to both mechanistic and diagnostic questions. It has also proved that it is feasible to undertake PET studies recruiting significant numbers of participants with FTD, emulating the direction of travel of *in vivo* studies in Alzheimer's disease. It has shown that the findings can be highly informative, not only providing *in vivo* corroboration of the presence of key pathophysiological processes, but also generating hypotheses which should be further examined in FTD. Importantly, it has also illustrated the limitations of using tools developed in one disease to study other, pathologically distinct, disease. A potential 'tau ligand' in FTD could have fulfilled a much needed role by classifying pathological diagnosis as tau or non-tau *in vivo*, and also could have provided insights in the toxicity of tau in FTD, similar to those enabled in Alzheimer's disease. Although potentially somewhat mitigated by the possibility of [¹⁸F]AV-1451 binding representing aggregation of tau or TDP-43 protein, i.e. as a marker of non- β -amyloid neuropathology in FTD, the failure of [¹⁸F]AV-1451 to provide this *in vivo* classification is unfortunate, but ultimately perhaps unsurprising, given the well-known and significant differences between subtypes of tau pathology at a biochemical, ultrastructural and morphological level. Ultimately, it is of the utmost importance that we develop improved, FTD-specific tools to examine the biology of disease *in vivo*. On the basis of the findings and lessons learned from this thesis, future *in vivo* PET studies in FTD should go forward to address some of the questions of timing, distribution, and toxicity which still need to be answered and which, after discussing the limitations of the project in more detail, I discuss below.

Limitations of this study

There are several limitations to this study which I will discuss individually below.

Recruitment and group composition

The individual diagnostic groups in this study are small. Whilst this is the result of these diseases being relatively rare it leads to limitations of power in the study and an increased chance of type 2 error. The groups are also inherently biased due to the strict inclusion criteria and the necessity to exclude individuals with floridly disinhibited symptoms or advanced disease who would be unable to complete the study protocol. A further source of bias is that the participants were recruited almost entirely from a single tertiary referral neurology clinic. The resultant diagnostic groups reflect the range of FTD seen in this setting but may not be truly representative of the FTD population in the community. The complexity of FTD also impacts on the purity of each diagnostic group. Although the groups are analysed on the basis of clinical syndrome they will also be pathologically heterogeneous. This is certainly the case in the bvFTD group where there are individuals with MAPT, GRN and C9orf72 mutations. *Ante mortem* pathological confirmation is not possible and so analysis on the basis of pathological diagnosis is not possible. We do not yet have *post mortem* validation of FTLN pathology as only one recruited participant has died and been examined in the brain bank. We also do not have β -amyloid status on FTD participants to exclude Alzheimer's pathology as a cause or concomitant feature of their disease.

Effects of atrophy on image processing

Marked atrophy is often a feature of FTD. This is an important consideration when processing the imaging data. In particular, significant atrophy, such as that seen in svPPA and in genetic forms of bvFTD, complicates spatial normalisation of MRI and co-registration to PET. Segmentation of MRI into grey and white compartments may also be compromised. Importantly, there is also an effect of atrophy on PET signal, resulting from contamination of patient voxels by an increased CSF fraction. To account for this partial volume correction is often undertaken in which the probabilistic contribution of CSF to a region of interest is calculated and used to adjust the binding potential. This is necessary to prevent binding being obscured by the degree of atrophy and essentially has the effect of pushing the observed binding into a smaller brain volume thereby inflating the PET value. This inflation is proportionate to the degree of atrophy and so is more marked in regions with severe atrophy. Given this high binding value coming from a small volume it seems reasonable to interpret the signal as highly likely to be coming from brain tissue with a high burden of pathology, particularly as atrophy is thought to be downstream of other pathophysiological processes

such as microglial activation and protein aggregation. Consequently, partial volume correction is accepted as standard practice in PET studies and has been used in all studies in this thesis.

Limitations of PET ligands

There are limitations regarding both PET ligands. [^{18}F]AV-1451 is less sensitive to neuropathology outside of Alzheimer's disease and whilst increased *in vivo* binding is seen the substrates of this are yet to be confirmed. Furthermore, *post mortem* studies thus far have shown minimal binding to FTLD-tau and FTLD-TDP43 species and so the biological relevance of this binding remains uncertain. Whilst the assumption that the anatomically appropriate binding in both tau and TDP-43 cases reflects *in vivo* binding to both tau and TDP-43 aggregates seems reasonable, it is a hypothesis which requires further investigation in order to be validated. What is more, other cells involved in neurodegeneration may upregulate certain proteins which may provide a binding site which though 'off-target' may be relevant to the pathophysiology of disease. For example, there is possible binding to monoamine oxidase expressed by reactive astrocytes in the vicinity of degenerating neurons (Barrio, 2018). A significant degree of truly 'off-target' binding, likely to pigments and minerals and unaffected by neurodegenerative processes, is also a feature of studies using [^{18}F]AV-1451. This is a particular problem in the basal ganglia and medial temporal lobe, regions which are relevant to FTD, leading to complications in interpreting the source of increased binding in these regions which likely suffer from disease effects but which also suffer from non-specific binding to pathological targets.

[^{11}C]PK-11195 also has limitations (Chauveau, Boutin, & Camp, 2008). It is less sensitive than newer TSPO radiotracers and although the effect of TSPO polymorphisms is less significant it is still likely to be present. This lack of sensitivity and potential inter-subject variability is a problem, potentially leading to poor signal to noise ratio and an inability to find effects which are present. Whichever TSPO ligand is studied, interpretation of results is also complicated by potential binding to upregulated TSPO on other cells involved in neurodegeneration such as reactive astrocytes. Our understanding of microglial TSPO expression patterns and functional outcomes from different microglial phenotypes is also incomplete and so whilst the presence of activated microglia may be assumed from increased binding the more subtle question of its effects cannot be addressed.

Aside from acknowledging these difficulties with both radiotracers, I have also tried to account for the common theme of variable sensitivity and signal to noise in the analysis by

using a multivariate approach, insensitive to absolute binding values and therefore to differences in sensitivities of tracers for different targets.

Statistical approaches

Finally I will address the approaches used in this thesis, some of which are non-standard. The purpose of the thesis has been to begin to answer some basic questions about pathophysiology *in vivo* in Frontotemporal dementia, and concomitantly about the tools used to study them. In every analysis I have used well established parametric methods to examine differences between groups and individuals or relationships between ligand binding. However, as discussed in the preceding chapters there are many variables which contribute to these findings, including patient factors, ligand factors and disease factors. These diseases are not subtle clinically or in the macroscopic structural changes they cause in the brain, however variables other than disease may affect magnitude of binding in a given participant and these may hide disease effects when assessed as a parametric comparison. Consequently, I adopted a different but complementary approach already in widespread use in neuroimaging, most commonly in Representational Similarity Analysis, an extremely powerful tool used in functional MRI where it is used to compare patterns of voxel activation in single subjects. In a similar way I use the individual's whole brain pattern of binding, blinded to the regional magnitude, to compare patients to healthy volunteers or those with distinct forms of Frontotemporal dementia. When using these methods to assess the difference or otherwise of the patient from controls, for example using hierarchical cluster analysis, there are a number of methods which may be used. The simplest is 'nearest neighbour', where one classifies individuals by serially finding the most similar distributions and ordering individuals by uniqueness. Under this method, the patient has the most unique distribution. However, as this thesis in part is about the potential utility of a novel biomarker, I generally give prominence to the most stringent method which, when comparing a single case to a control group, is 'farthest neighbour'. In this method, individuals are placed into groups based on how dissimilar they are from the most dissimilar member of that group. In the case of a single case, for perfect separation this would require all of the controls to be extremely homogenous in their distributions. Given that the correlation analyses are non-parametric and that control PET ligand binding is low and relatively uniform, this is unlikely. Therefore, the classification of the case as separate from the controls using this most conservative method provides strong evidence for the ability of the ligand to illustrate a distribution of binding which is different from the 'normal' pattern seen in controls and which may be interpreted as disease specific

given the anatomical location of the regional differences seen in the parametric group comparisons.

Future research and potential applications

This thesis provides evidence, both generally for the idea of using PET ligands to examine specific components of neurodegenerative pathophysiology *in vivo*, and more specifically for the role of both protein aggregation and neuroinflammation during FTD. However, there are many important questions regarding these processes which cannot be answered by this study and which warrant further investigation, as well as other pathophysiological processes which may interact with neuroinflammation or protein aggregation. Many of these basic questions about pathophysiology need to be answered to improve our *in vivo* understanding of the biology of disease in FTD and to determine whether specific components of pathophysiology may be targets for therapeutic intervention. Here I will suggest 4 important questions regarding neuroinflammation to be addressed in the future and how they might be approached.

1) Is the timing of neuroinflammation or protein aggregation early or late and does it continue throughout the disease process?

We began to try to address this question by examining both processes in a pre-symptomatic gene carrier. Larger studies of a similar nature will be required but also with longitudinal clinical and imaging follow-up to assess onset, tempo and pattern of these processes and their relationship to clinical phenotype and severity.

2) Is the effect of neuroinflammation protective, deleterious or does it change from one to the other at different time points in the illness?

This is linked to the above question and is difficult to answer as neuroinflammation might be a characteristic of the individual as much as the disease (Hamelin, Lagarde, Potier, et al., 2018). Large cohorts would need to be recruited early in disease, including pre-symptomatic gene carriers, and stratified according baseline inflammatory status. Progression both clinically and in inflammation could then be assessed. An independent disease group with moderate disease could be stratified by baseline inflammation and again by impact on clinical severity and longitudinal progression of inflammation then assessed enabling comparison between the presence and progression of inflammation at early and established stages of disease.

3) Is neuroinflammation a feature of particular subtypes of pathology or is it a common mechanism across the spectrum of FTL?

Cohorts of genetic FTD, where pathology is tightly correlated with genetic aetiology, and which may act as models of their sporadic equivalents (Forrest et al., 2018; Josephs, 2018). The use of PET in larger cohorts of genetic FTD will be of great interest, but the evidence from the sporadic and genetic cases presented here, it seems likely that all types of FTD are associated with elevated neuroinflammation. What is less clear is the temporal and causal relationship between inflammation and protein aggregation or cell death.

4) How does neuroinflammation or protein aggregation relate to other relevant brain metrics, e.g. those related to information transfer such as network function and synaptic density?

Answering this question will require multimodal studies which enable the relationship of pathophysiology to network function and clinical status. This issue has started to be addressed in studies such as GENFI which have looked at the role of networks in pre-symptomatic gene carriers. Ideally this could be extended by evaluating neuroinflammation, protein aggregation and other components such as synaptic density in a similar cohort and also in those with established non-genetic FTD to examine associations of key pathophysiological processes with network function and clinical phenotype pre and post disease onset.

Conclusion

The successful proof of concept studies of genetic FTD, and the larger cohort study of symptomatic FTD, outlined in this thesis suggest that PET is useful to quantify and localise and stage pathologies. However, these studies also raise new questions about causality and heterogeneity that call for larger and longitudinal cohorts with multimodal imaging and in due course therapeutic interventions.

PET imaging is required with ligands specific to protein pathologies (some of which have yet to be developed e.g. for TDP-43 and α -synuclein), as well as improved markers of neuroinflammation with insensitivity to polymorphisms in TSPO, better signal to noise ratios and high specific binding to activated microglia, or potentially to other components of the neuroinflammatory cascade. Radioligands for other pathophysiological components such as synaptic density (which for example can be measured using the newly developed UCB-J ligand) would also be informative about the cascade of targetable events leading from root genetic and environmental causes to complex phenotypes. These ligands will also require robust non-invasive models for calculating binding which do not require arterial blood sampling or lengthy scans so that large numbers of participants, including frail and elderly

people with advanced disease, as well as the more behaviourally disturbed, may be able to take part in these studies. As well as including participants with svPPA, nvPPA and bvFTD, these studies should also enrol pre-symptomatic gene carriers with each of the 3 major genes associated with FTD.

The potential power of large-scale studies of pre-symptomatic gene carriers has already been illustrated by GENFI and the addition of PET may provide pathophysiological insights which transcend genetic FTD given that recent models of neurodegeneration in FTD suggest that genetic tauopathies may represent a model of sporadic FTLD-tau disease and similarly for TDP-43 cases (Forrest et al., 2018; Josephs, 2018). Longitudinal studies extending from pre-symptomatic states right through the clinical spectrum of disease will be necessary to determine the timing of inflammation, its relationship to other pathological process such as protein aggregation, neuronal loss, synaptic density and network function, and how these features and their relationships pertain to clinical phenotype, disease severity and progression.

My goal is that improved radioligands could be used in clinical trials of drugs targeting specific mechanisms, either as stratifying inclusion criteria, surrogate end-points or both. It is my hope that the work in this thesis will enable the field to begin to move beyond phenomenology and towards the biological definition of these diseases so that we can ultimately design and implement successful clinical trials and bring to an end the enormous health, economic and above all social burden of frontotemporal dementia.

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Appendix 1

Neuropsychology battery

Test Name	Format	Purpose	Cohort
<i>Clinical Assessment</i>			
UPDRS Part III (motor subscale) (Goetz et al., 2008)	Performed by study clinician	Measure of Parkinsonism (motor aspects)	All
Physical Examination of Eye Movements	Part of physical examination performed by study clinician	Assessment of range and speed of eye movements	All
Frontal Assessment Battery (Slachevsky, 2000)	Assessment tool completed by study clinician	Assessment of frontal lobe function	FTD, PSP
Praxis Battery	Part of physical examination performed by study clinician	Assessment of manual ideomotor and copying ability	All
<i>Neuropsychological Assessment</i>			
Addenbrooke's Cognitive Examination – Revised (Eneida Mioshi et al., 2006)	Researcher administered structured test	Multi-domain cognitive screening tool	All
INECO Frontal Screening (Torralva, Roca, Gleichgerrcht, López, & Manes, 2009)	Researcher administered structured test	Assessment of frontal lobe function	All
Trails A & B	Researcher administered structured test	Assessment of executive function	All
Pyramids and Palm Trees	Researcher administered, two alternative, forced choice, picture based test	Assessment of semantic memory	All
<i>Cambridge Neuropsychological Test Automated Battery (CANTAB)</i>			
Simple Reaction Time (http://www.cambridgecognition.com/tests/simple-reaction-time-srt/ , n.d.)	Researcher administered computer task	Information processing speed	All

Paired Associate Learning (Swainson et al., 2001)	Researcher administered computer task	Assessment of visual episodic memory and learning	All
Stockings of Cambridge (Robbins et al., 1998)	Researcher administered, computer based spatial planning task	Test of frontal lobe function	All
<i>Mental Health Questionnaires</i>			
Hospital Anxiety & Depression Scale	A 14 item self-reported questionnaire	Assessment of symptoms of anxiety and depression	All
Geriatric Depression Scale	A 30 item self-reported questionnaire	Assessment of depressive symptoms	All
<i>Informant Questionnaires</i>			
Cambridge Behavioural Inventory (Wedderburn et al., 2008)	An 81 item carer-reported questionnaire	Assessment of several behavioural abnormalities in the everyday life including impulsivity and apathy	All except controls
Frontotemporal dementia Rating Scale	A 30 item carer-reported questionnaire	Assessment of behaviour and functional abilities	FTD only
Clinical Dementia Rating Scale (Hughes et al., 1982)	A carer-reported numerical scale	Quantifying severity of dementia	All except controls
Bristol Activities of Daily Living Score	A 20 item carer-reported questionnaire	Measure of ability of person with dementia to carry out ADLs	All except controls
Neuropsychiatric Inventory (Cummings et al., 1994)	Researcher administered, carer-reported 13 item screening tool	Assessment of psychopathology in people with brain disorders	All except controls
Clinical Assessment of Fluctuating Confusion and Quality of Consciousness (M. P. Walker et al., 2000)	Researcher administered, carer-reported 9 item screening tool	Assessment of conscious level and degree of symptomatic arousal fluctuation	All except controls

MRI Imaging

MRI scanning was performed at the WBIC using 3 Tesla Siemens scanners. The following sequences were performed during the scanning protocol:

1. Three-dimensional structural high-resolution T1 weighted sequence examining for structural brain abnormalities (176 slices of 1.0 mm thickness, TE= 2.98 ms, TR = 2300 ms, flip angle =9°, acquisition matrix 256x240; voxel size = 1x1x1 mm³).
2. Perfusion (pulsed arterial spin labelling) for blood flow (9 slices of 8.0 mm thickness, TE = 13 ms, TR = 2500 ms, acquisition matrix 64x64; voxel size = 4x4x8 mm³, inversion time 1 = 700 ms, inversion time 2 = 1800 ms).
3. Diffusor Tensor Imaging (DTI) to obtain fractional anisotropy measures of white matter integrity and gross axonal organisation (63 slices of 2.0 mm thickness, 63 diffusion directions, TE = 106 ms, TR = 11700 ms, b-value 1 = 0 s/mm², b-value 2 = 1000 s/mm², acquisition matrix 96x96; voxel size =2x2x2 mm³).
4. Susceptibility weighted imaging (SWI) to identify microhaemorrhages, venous blood and iron deposition (40 slices of 2.0 mm thickness, TE = 20 ms, TR = 35 ms, flip angle = 17°, acquisition matrix 256x240; voxel size =1x1x2 mm³).
5. High resolution hippocampal subfield sequences carried out in coronal T2 for smaller structural changes in the hippocampus (20 slices of 2.0 mm thickness, TR = 6420 ms, flip angle = 160°, acquisition matrix 512x408; voxel size =0.4x0.4x2 mm³).
6. Resting state functional MRI with pulse and breathing monitored to examine “task-free” functional brain connectivity (34 slices of 3.8 mm thickness, TE = 13 ms, TR = 2430 ms, flip angle = 90°, acquisition matrix 64x64; voxel size =3.8x3.8x3.8 mm³, duration 11 minutes and 5 seconds).
7. T2 Fluid Attenuated Inversion Recovery (FLAIR) for characterising periventricular lesions adjacent to the sulci and white matter lesions and hyperintensities (75 slices of 2 mm thickness, TE = 132 ms, TR = 12540 ms, flip angle = 120°, acquisition matrix 256x256; voxel size =0.9x0.9x2 mm³).

Blood Samples

80ml of blood was drawn at the time of the [¹¹C]PK-11195 PET scan. Basic inflammatory markers (e.g. C-reactive protein) were tested at the time of the sample with the remaining blood being centrifuged to produce serum and plasma samples to be stored at -80°C for future testing of inflammatory and neurodegenerative markers. A sub group of participants also had

blood immuno-phenotyping based on the availability of the technique to the study. These participants had blood samples analysed on the same day using flow cytometry for immune-phenotyping.

CSF Samples

For those who consented to have CSF collected, samples were obtained on a separate study visit. The samples (up to 10ml) were obtained by a lumbar puncture and a sample sent to the laboratory at UCL for quantification of total tau and amyloid- β -42. The remainder of the sample was centrifuged and stored at -80°C for future testing of inflammatory and neurodegenerative markers.

Consent Form for Control Subjects

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

I,(name)

of

.....(address)

consent to taking part in the NIMROD study.

Please tick

I have read the information sheet giving details of this study, have been given a copy to keep and have had the opportunity to ask questions.

☐

I understand that my participation is voluntary and I can withdraw consent at any time without giving any reason and without my medical care or legal rights being affected.

☐

I understand that data collected during the study may be looked at in the monitoring of the research by clinical governance staff. I give permission for such access to my records.

☐

I understand that my tissue samples will be tested for genetic factors and I agree to this.

☐

I give permission for information concerning me to be held by the University of Cambridge. I understand that records will be confidential and will be stored securely on systems within the NHS and University.

☐

I give my permission that in the unlikely event that an abnormality is discovered my GP and I will be informed.

☐

I consent for my data and tissue samples to be used in similar studies.

☐

Signed..... **Date**

Consented by(sign) **Date**

Print name.....

When completed, original to be kept in research file, 1 copy for Participant

Consent Form for Informants

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

I,(name)

of

.....(address)

consent to taking part in the NIMROD study by answering questions and completing questionnaires and other scales relating to the capabilities and behaviour of

[Name]..... “the
Participant”

Please tick

I have read the information sheet giving details of this study, have been given a copy to keep and have had the opportunity to ask questions.

☐

I understand that my participation is voluntary and I can withdraw consent at any time without giving any reason and without the Participant’s medical care or legal rights being affected.

☐

I understand that data collected during the study may be looked at in the monitoring of the research by clinical governance staff. I give permission for such access to the information I provide.

☐

I give permission for the information I provide to be held by the University of Cambridge. I understand that records will be confidential and will be stored securely on systems within the NHS and University.

☐

I consent for the information I provide to be used in similar studies and in connection with the medical treatment and care of the Participant.

☐

Signed..... **Date**

Consented by(sign) **Date**

Print name.....

When completed, original to be kept in research file, 1 copy for Informant, 1 copy for Participant

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

I, (name)

of

..... (address)

consent to taking part in the NIMROD study.

Please tick

I have read the information sheet giving details of this study, have been given a copy to keep and have had the opportunity to ask questions.

☐

I understand that my participation is voluntary and I can withdraw consent at any time without giving any reason and without my medical care or legal rights being affected.

☐

I understand that sections of any of my medical notes may be looked at and information taken from them used in this research, or in the monitoring of the research by clinical governance staff. I give permission for the researchers and clinical governance staff to have such access to my records.

☐

I understand that my tissue samples will be tested for genetic factors and I agree to this.

☐

I give permission for information concerning me to be held by the University of Cambridge. I understand that records will be confidential and will be stored securely on systems within the NHS and University.

☐

I understand that my GP will be informed of my participation in this study, and give permission for this.

☐

I give my permission that in the unlikely event that an abnormality is discovered my GP and I will be informed.

☐

In the possible event of my losing mental capacity to give informed consent during this study, I wish it to be noted that I am minded to continue in the study.

☐

I consent for my data and tissue samples to be used in similar studies.

☐

Signed..... Date

Consented by(sign) **Date**

Print name.....

When completed, original to be kept in research file, 1 copy for Participant

Declaration Form for Consultees

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

I, (name)
of
..... (address)

am acting on behalf of my relative/dependant/friend in my capacity as their personal consultee as defined by the Mental Capacity Act (2005) with the understanding that my relative/dependant/friend does not have capacity to decide to participate in this research study for him/herself.

I have been consulted about (name)
taking part in the NIMROD study.

Please tick

I have read the information sheet giving details of this study and I have had the opportunity to ask questions and understand what is involved.

☐

In my opinion he/she would not object to taking part in the NIMROD study.

☐

I understand that participation is voluntary and that I can request he/she is withdrawn from the study at any time without giving any reason and without their medical care or legal rights being affected.

☐

I understand that relevant sections of my relative/dependant/friend's medical notes may be looked at by the research team and information taken from them for use in this research, or in the monitoring of the research by clinical governance staff.

☐

I understand that my relative/dependant/friend's tissue samples will be tested for genetic factors and in my opinion he/she would agree to this.

☐

I understand that information concerning my relative/dependant/friend will be held by the University of Cambridge. I understand that records will be confidential and will be stored securely on systems within the NHS and University.

☐

I understand that my relative/dependant/friend's GP will be informed of their participation in this study.

☐

I understand that in the unlikely event that an abnormality is discovered my relative/dependant/friend and his/her GP will be informed.

☐

I agree that my relative/dependant/friend's data and tissue samples can be used in future similar studies.

☐

Signed..... (Consultee) **Date**

Relationship to Patient.....

Consulted by..... (Team member) **Date**

Print name.....

When completed, original to be kept in care record, 1 copy for consultee and 1 copy for research file

Information Sheet for Consultees

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

[Name]..... is being invited to take part in a research study. We feel he/she is unable to decide for himself/herself whether to do so. We would like your opinion whether or not they would like to be involved. This leaflet explains why the research is being done and what taking part will involve. Please read the following information carefully and discuss it with others if you wish. Please consider what you know of their wishes and feelings, and consider their best interests. It will help if you can let us know of any advance decisions they have made about participating in research as these would take precedence. You can talk to the researchers before you decide.

If you decide that they would have no objection to taking part, after reading this information leaflet, please sign the consultee declaration form. We will keep you both fully informed during the study so you can let us know if you have any concerns or you think your relative/dependant/friend should be withdrawn from further participation.

If you decide they would not wish to take part it will not affect the standard of health care they receive in any way.

If you are unsure about taking the role of consultee you may seek independent advice; we understand if you don't want to take on this responsibility. The following information is the same as we provide for people who are able to consent for themselves.

What is the purpose of the study?

There are several different causes of memory problems in later life, including a condition called Mild Cognitive Impairment as well as different types of dementia like Alzheimer's disease, Frontotemporal dementia, Lewy body dementia, Progressive supranuclear palsy and Vascular dementia. Older people with depression can also have memory problems.

While considerable progress has been made over the last decade in understanding some of the brain protein and other changes associated with memory problems and dementia, a lot is still not known. For example, why some people with memory problems get worse at a faster rate than others is not clear. It has been established that mild inflammatory changes (brain

inflammation) are associated with some of these disorders, but the questions whether it is present in all of them, and if so precisely where and how it changes with time have received little research attention. This is important as we do not know how much inflammation is the result of disease and how much it may be involved as a cause. If it is a cause, then this is extremely important because it may be possible to develop new treatments to help prevent memory problems getting worse. It may also be possible to use measures of inflammation to predict groups of individuals who may be more at risk of declining more quickly than others.

Brain imaging is an important method to investigate brain structure and function. Magnetic Resonance Imaging (MRI) can be used to look at brain structure and function in great detail. In addition, Positron Emission Tomography (PET) imaging can be used to help visualise some kinds of damaged protein often found in people with memory and other problems (amyloid using PIB PET and tau using AV-1451 PET), and also can help detect the presence of inflammation in the brain (PK11195 PET). Further, the presence of illnesses, including inflammation, leaves tell-tale changes in the blood and cerebro-spinal fluid, the clear fluid that bathes the brain and spinal cord. In addition there are known to be genetic markers found in the blood that are associated with increased or decreased risk of dementia.

This study looks for the presence of damaged protein, genetic markers, inflammation and changes in brain structure and function in people with a range of disorders that affect their thinking, attention and memory as well as suitable control subjects without such impairments. We will compare them to see how they differ on the tests and scans to understand the causes and effects of dementia and related illnesses.

Why has your relative/dependant/friend been invited?

Your relative/dependant/friend has been selected either because he/she has been diagnosed with a neurodegenerative disease or with depression or because he/she has symptoms that are suggestive of such a disorder.

Does my relative/dependant/friend have to take part?

It is up to your relative/dependant/friend to decide whether to join the study. We will rely on your guidance to understand his or her wishes in this regard.

If you decide he/she would not wish to take part it would not affect the standard of health care he/she receives in any way.

If you feel that he/she would agree to take part we will ask you to sign a consent form.

He/she is free to withdraw at any time without giving a reason. This would not affect the standard of health care he/she receives in any way.

What will your relative/dependant/friend be asked to do?

The study includes the following types of test, although not everyone will necessarily be asked to do all parts of the study:

1. A clinical assessment including tests of memory, words, vision etc
2. A blood test.
3. An MRI brain scan
4. Either one or two PET scans

Participants will have tests of memory, language, vision and attention, which take about one and a half hours to complete. This would be repeated every year for the duration of the study (up to 3 years) and can be carried out either at your relative/dependant/friend's home or here at Addenbrooke's Hospital, whichever they and you prefer. We may use an audio device to record some of their answers. Recordings will only be used as a supplement to written notes to ensure accuracy and will be deleted after use.

We will ask you or someone else who knows them well to have a short interview to answer questions and complete a couple of questionnaires about how he or she is coping with everyday life.

We would carry out a brief physical examination which could either be as part of a normal clinical attendance or combined with one of the other research visits. This would be repeated annually. We would also take a blood sample (about 80 ml, or 2 eggcups full). These take about 10 minutes.

We propose to undertake up to two PET scans at Addenbrooke's Hospital in the Wolfson Brain Imaging Centre (WBIC). On each occasion your relative/dependant/friend will be in WBIC for approximately 2 hours, with the scan itself taking 45 minutes in the case of PIB PET and 90 minutes for the other (PK11195 and AV-1451 PET). For each PET scan they will have an injection of about a teaspoon of short lasting radioactive liquid. The radiation dose for each PIB and PK11195 PET scan (2.7 – 3.0 milliSieverts) is similar to the radiation dose we each experience from radiation in the environment during one year living in the East Anglia region, while the radiation dose from AV-1451 PET is 9.3 milliSieverts, which is similar to 3½ years' environmental exposure here. The injected radioactivity fades away

naturally over a few hours and your relative/dependant/friend can leave the WBIC as soon as the scan is finished.

In all cases our staff would communicate with them throughout the scan to check that they stay comfortable. You or your relative/dependant/friend could end the scan at any point.

The MRI brain scan also takes place at the WBIC. This is to look at the size, shape and 'wiring' of the brain. It may be possible to arrange for this to take place on the same day as one of the PET scans. Though MRI scanning is generally very safe, there are certain circumstances when it must be avoided. We will go through a checklist to ask about metal objects attached to or inside his or her body (e.g. stents, shrapnel, plated fractures,) or electronic devices (e.g. heart pace-maker). Many such items (most modern cardiac stents, for instance) are designed to be MRI safe. Being scanned requires the participant to lie still and relaxed on a bed in the scanner's 'tunnel'. This 'tunnel' is quite narrow so please let us know if your relative/dependant/friend is likely to suffer feelings of claustrophobia there. It can be noisy but earplugs are supplied and the participant can also have their own choice of music played over headphones if they wish. As with the PET scans, the technician performing the MRI scan would communicate with them throughout the scan to check that they stay comfortable. It can be stopped at any point, but takes up to one hour to complete.

What are the possible benefits of taking part?

This is not a trial of any drug or other treatment and there is no direct benefit to you or your relative/dependant/friend from taking part in this study. However if he/she does take part he/she will be making a significant contribution to medical knowledge and the challenge of dementia especially.

Expenses

If your relative/dependant/friend takes part in this study, we would cover all necessary travel expenses and if it would help we would arrange transport by taxi for your relative/dependant/friend (and you if you like) to come to the hospital and go home.

Will my relative/dependant/friend's taking part in the study be kept confidential?

If your relative/dependant/friend does take part in the study, all information provided to us and the results of studies would be treated confidentially. It will be stored securely in locked cabinets or on password protected computer systems under the supervision of the Chief Investigators. We will retain the data for over 10 years. We will ask for your permission on behalf of your relative/dependant/friend to share their data and scans in an anonymised way with collaborators, now and in the future, including researchers in the NHS, Medical Research

Council, University and National Institute for Health Research. The NHS is trying to improve the quality of clinical and research standards. This is being achieved through ‘clinical governance’. As part of this process, this study may be reviewed by a clinical governance team. Such a team would need to look at our records to make sure that the research was carried out in accordance with proper procedures.

What if there is a problem?

Although the PET scans are for research purposes only, the MRI scan will be routinely reported by a specialist radiologist. Occasionally, brain scanning and other tests reveal a medical problem that was not expected. If this happens, we will inform you and your relative/dependant/friend, and (if you agree on their behalf) we would write to their General Practitioner (GP) and arrange for any necessary NHS follow up.

We have also arranged insurance, in the unlikely event of any problems, without affecting your relative/dependant/friend’s statutory rights. If you have any concerns or comments related to your relative/dependant/friend’s participation in this study, you could contact the Chief investigator (details below) or the Patient Advisory and liaison Service (PALS) at Box 53, Cambridge University Hospitals, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0QQ, telephone 01223 216 756, e-mail pals@addenbrookes.nhs.uk .

Who is organising and funding the research?

The study is funded primarily by the NIHR (National Institute for Health Research) Biomedical Research Unit. The research team are based at the Departments of Psychiatry and Clinical Neurosciences at the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust (Addenbrooke’s Hospital)

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect the participants’ interests. This study has been reviewed and given a favourable opinion by the East of England - Cambridge Central Research Ethics Committee.

Further information

If you would like further information please contact Professor John O’Brien, or any member of the research team (details below).

What will happen next?

The next step will be a telephone call from one of the researchers. If you are interested in helping with the study, they will arrange to visit you at home. This will give you a chance to ask any questions about the study and your relative/dependant/friend taking part before you make a decision. If you do advise on behalf of your relative/dependant/friend that they should take part, the researcher will discuss a consultee declaration form with you and ask you to sign it. It is up to you to decide whether to advise that your relative/dependant/friend should take part or not. You should base this decision on your understanding of what your relative/dependant/friend would have decided had they had the mental capacity to do so. You do not have to give a reason if you advise that your relative/dependant/friend should not be involved. Whatever you decide will have no effect on the care your relative/dependant/friend receives now or in the future. If you change your mind on behalf of your relative/dependant/friend you can advise that they withdraw from the study at any time without giving a reason. You will be given a copy of this leaflet to keep.

The research team should contact you in the next week or so. If, at any time, you need to get in touch with someone, you can contact us as shown below:

Professor John T O'Brien (Chief Investigator),
Department of Psychiatry,
University of Cambridge,
Level E4, Box 189,
Addenbrooke's Hospital,
Hills Road,
Cambridge, CB2 0SP
Tel: +44 (0)1223 760682

Dr James B Rowe (co-Chief Investigator),
Department of Clinical Neuroscience,
University of Cambridge,
Herchel Smith Building,
Forvie Site,
Robinson Way,
Addenbrooke's Hospital ,
Cambridge, CB2 0SZ
Telephone: +44 (0) 1223 273 630
Secretary: +44 (0) 1223 760 696

Robert Arnold (Research Assistant)
Herchel Smith Building,
Forvie Site, Robinson Way,
Cambridge, CB2 0SZ
Telephone: 01223 768003
Email: rja39@medschl.cam.ac.uk

Information Sheet for Control Participants

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

You are invited to take part in a research study. This leaflet explains why the research is being done and what taking part will involve. Please read the following information carefully and discuss it with others if you wish. You can talk to the researchers before you decide. If you decide to take part, after reading this information leaflet, please sign the consent form.

What is the purpose of the study?

There are several different causes of memory problems in later life, including a condition called Mild Cognitive Impairment as well as different types of dementia like Alzheimer's disease, Frontotemporal dementia, Lewy body dementia, Progressive supranuclear palsy and Vascular dementia. Older people with depression can also have memory problems.

While considerable progress has been made over the last decade in understanding some of the brain protein and other changes associated with memory problems and dementia, a lot is still not known. For example, why some people with memory problems get worse at a faster rate than others is not clear. It has been established that mild inflammatory changes (brain inflammation) are associated with some of these disorders, but the questions whether it is present in all of them, and if so precisely where and how it changes with time have received little research attention. This is important as we do not know how much inflammation is the result of disease and how much it may be involved as a cause. If it is a cause, then this is extremely important because it may be possible to develop new treatments to help prevent memory problems getting worse. It may also be possible to use measures of inflammation to predict groups of individuals who may be more at risk of declining more quickly than others.

Brain imaging is an important method to investigate brain structure and function. Magnetic Resonance Imaging (MRI) can be used to look at brain structure and function in great detail. In addition, Positron Emission Tomography (PET) imaging can be used to help visualise some kinds of damaged protein often found in people with memory and other problems (amyloid using PIB PET imaging and tau using AV-1451 PET imaging), and also can help

detect the presence of inflammation in the brain (PK11195 PET). Further, the presence of illnesses, including inflammation, leaves tell-tale changes in the blood and cerebro-spinal fluid, the clear fluid that bathes the brain and spinal cord. In addition there are known to be genetic markers found in the blood that are associated with increased or decreased risk of dementia.

This study looks for the presence of damaged protein, genetic markers, inflammation and changes in brain structure and function in people with a range of disorders that affect their thinking, attention and memory. To do this we also need to study control subjects such as yourself without such impairments. We will compare them to see how they differ on the tests and scans, to understand the causes and effects of dementia and related illnesses.

Why have I been invited?

You have been chosen as a healthy person, so we can compare our tests between normal people and patients with dementia and other illnesses.

Do I have to take part?

It is up to you to decide whether to join the study. If you decide you do not wish to take part it would not affect the standard of health care you receive in any way.

If you agree to take part we will ask you to sign a consent form.

You are free to withdraw at any time without giving a reason. This would not affect the standard of health care you receive in any way.

What will I be asked to do?

The study includes the following types of test, although not everyone will necessarily be asked to do all parts of the study:

1. A clinical assessment, including memory and other cognitive tests
2. A blood test.
3. An MRI brain scan
4. Either one or two PET scans
5. Some people may also be invited for a lumbar puncture, to examine spinal fluid.

Participants will have tests of memory, language, vision and attention, which take about one and a half hours to complete. This would be repeated every year for the duration of the study (up to 3 years) and can be carried out either at your home or here at Addenbrooke's Hospital, whichever you prefer. We may use an audio device to record some of your answers.

Recordings will only be used as a supplement to written notes to ensure accuracy and will be deleted after use.

We would carry out a brief physical examination which could be combined with one of the other research visits. This would be repeated annually. We would also take a blood sample (about 80 ml, or 2 eggcups full). These take about 10 minutes.

We propose to undertake up to two PET scans at Addenbrooke's Hospital in the Wolfson Brain Imaging Centre (WBIC). On each occasion you will be in WBIC for approximately 2 hours, with the scan itself taking 45 minutes in the case of PIB PET and 90 minutes for the other (PK11195 PET). For each PET scan you will have an injection of about a teaspoon of short lasting radioactive liquid. The radiation dose for each PET scan (2.7 – 3.0 milliSieverts) is similar to the radiation dose we each experience from radiation in the environment during one year living in the East Anglia region. The injected radioactivity fades away naturally over a few hours and you can leave the WBIC as soon as the scan is finished.

In all cases our staff would communicate with you throughout the scan to check that you stay comfortable. You could end the scan at any point.

The MRI brain scan will take around an hour and also takes place at the WBIC. This is to look at the size, shape and 'wiring' of the brain. It may be possible to arrange for this to take place on the same day as one of the PET scans. Though MRI scanning is generally very safe, there are certain circumstances when it must be avoided. We will go through a checklist to ask about metal objects attached to or inside your body (e.g. stents, shrapnel, plated fractures,) or electronic devices (e.g. heart pace-maker). Many such items (most modern cardiac stents, for instance) are designed to be MRI safe. Being scanned requires you to lie still and relaxed on a bed in the scanner's 'tunnel'. This 'tunnel' is quite narrow so please let us know if you have experienced claustrophobia in small spaces. It can be noisy but earplugs are supplied and you can also have your own choice of music played over headphones if you wish. As with the PET scans, the technician performing the MRI scan would communicate with you throughout the scan to check that you stay comfortable. It can be stopped at any point, but takes up to one hour to complete.

Some participants would also be invited for a lumbar puncture, on another visit, to take a small volume (about 15ml, three teaspoonsful) of the spinal fluid that has bathed the brain before travelling down the spine. It can tell us a lot about what is happening in the brain. A separate information sheet is available on lumbar puncture, as it is not relevant to everyone, and is an optional part of the study.

What are the possible benefits of taking part?

This is not a trial of any drug or other treatment and there is no direct benefit to you from taking part in this study. However if you do take part you will be making a significant contribution to medical knowledge and the challenge of dementia especially.

Expenses

If you take part in this study, we would cover all necessary expenses.

Will my taking part in the study be kept confidential?

If you do take part in the study, all information provided to us and the results of studies would be treated confidentially. It will be stored securely in locked cabinets or on password protected computer systems, under the supervision of the Chief investigators. We will retain the data for over 10 years. We will ask for your permission to share your data and scans in an anonymised way with collaborators, now and in the future, including researchers in the NHS, Medical Research Council, University and National Institute for Health Research. The NHS is trying to improve the quality of clinical and research standards. This is being achieved through 'clinical governance'. As part of this process, this study may be reviewed by a clinical governance team. Such a team would need to look at our records to make sure that the research was carried out in accordance with proper procedures.

What if there is a problem?

Although the PET scans are for research purposes only, the MRI scan will be routinely reported by a specialist radiologist. Occasionally, brain scanning and other tests reveal a medical problem that was not expected. If this happens, we will inform you, and (if you agree) we would write to your General Practitioner (GP) and arrange for any necessary NHS follow up.

We have also arranged insurance, in the unlikely event of any problems, without affecting your statutory rights. If you have any concerns or comments related to your participation in this study, you could contact the Chief Investigator (details below) or the Patient Advisory and Liaison Service (PALS) at Box 53, Cambridge University Hospitals, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0QQ, telephone 01223 216 756, e-mail pals@addenbrookes.nhs.uk.

Who is organising and funding the research?

The study is funded primarily by the NIHR (National Institute for Health Research) Biomedical Research Unit. The research team are based at the Departments of Psychiatry and Clinical Neurosciences at the University of Cambridge and Cambridge University Hospitals NHS Foundation Trust (Addenbrooke's Hospital)

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect the participants' interests. This study has been reviewed and given a favourable opinion by the East of England – Cambridge Central Research Ethics Committee.

Further information

If you would like further information please contact Professor John O'Brien, or any member of the research team (details below).

What will happen next?

The next step will be a telephone call from one of the researchers. If you are interested in helping with the study, they will arrange to visit you at home. This will give you a chance to ask any questions about the study and your taking part before you make a decision. If you do decide to take part, the researcher will discuss a consent form with you and ask you to sign it. It is up to you to decide whether to take part or not. You do not have to give a reason if you decide not to be involved. If you change your mind you can withdraw from the study at any time without giving a reason. You will be given a copy of this leaflet to keep.

The research team should contact you in the next week or so. If, at any time, you need to get in touch with someone, you can contact us:

Professor John T O'Brien (Chief Investigator)
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Robert Arnold (Research Assistant)
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Information Sheet for Patients and their Informants

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

You are being invited to take part in a research study. This leaflet explains why the research is being done and what taking part will involve. Please read the following information carefully and discuss it with others if you wish. You can talk to the researchers before you decide.

If you decide to take part, after reading this information leaflet, please sign the consent form.

If you decide not to take part it will not affect the standard of health care you receive in any way.

What is the purpose of the study?

There are several different causes of memory problems in later life, including a condition called Mild Cognitive Impairment as well as different types of dementia like Alzheimer's disease, Frontotemporal dementia, Lewy body dementia, Progressive supranuclear palsy and Vascular dementia. Older people with depression can also have memory problems.

While considerable progress has been made over the last decade in understanding some of the brain protein and other changes associated with memory problems and dementia, a lot is still not known. For example, why some people with memory problems get worse at a faster rate than others is not clear. It has been established that mild inflammatory changes (brain inflammation) are associated with some of these disorders, but the questions whether it is present in all of them, and if so precisely where and how it changes with time have received little research attention. This is important as we do not know how much inflammation is the result of disease and how much it may be involved as a cause. If it is a cause, then this is extremely important because it may be possible to develop new treatments to help prevent memory problems getting worse. It may also be possible to use measures of inflammation to predict groups of individuals who may be more at risk of declining more quickly than others.

Brain imaging is an important method to investigate brain structure and function. Magnetic Resonance Imaging (MRI) can be used to look at brain structure and function in great detail.

In addition, Positron Emission Tomography (PET) imaging can be used to help visualise some kinds of damaged protein often found in people with memory and other problems (amyloid using PIB PET imaging and tau using AV-1451 PET imaging), and also can help detect the presence of inflammation in the brain (PK11195 PET). Further, the presence of illnesses, including inflammation, leaves tell-tale changes in the blood and cerebro-spinal fluid, the clear fluid that bathes the brain and spinal cord. In addition there are known to be genetic markers found in the blood that are associated with increased or decreased risk of dementia.

This study looks for the presence of damaged protein, genetic markers, inflammation and changes in brain structure and function in people with a range of disorders that affect their thinking, attention and memory as well as suitable control subjects without such impairments. We will compare them to see how they differ on the tests and scans, to understand the causes and effects of dementia and related illnesses.

Why have I been invited?

You have been selected because you have either been diagnosed with a neurodegenerative disease or with depression or because you have symptoms that are suggestive of such a disorder.

Do I have to take part?

It is up to you to decide whether to join the study. If you decide you do not wish to take part it would not affect the standard of health care you receive in any way.

If you agree to take part we will ask you to sign a consent form.

You are free to withdraw at any time without giving a reason. This would not affect the standard of health care you receive in any way.

What will I be asked to do?

The study includes the following types of test, although not everyone will necessarily be asked to do all parts of the study:

1. A clinical assessment, including memory and other cognitive tests.
2. A blood test.
3. An MRI brain scan
4. Either one or two PET scans
5. Some people may also be invited for a lumbar puncture, to examine spinal fluid.

Participants will have tests of memory, language, vision and attention, which take about one and a half hours to complete. We will ask someone who knows you well to have a short

interview to answer questions and complete a couple of questionnaires about how you are and how you are coping with everyday life. This would be repeated every year for the duration of the study (up to 3 years) and can be carried out either at your home or at Addenbrooke's Hospital, whichever you prefer. We may use an audio device to record some of your answers. This will only be used as a supplement to written notes to ensure accuracy.

We would carry out a brief physical examination which could either be as part of a normal clinical attendance or combined with one of the other research visits. This would be repeated annually. We would also take a blood sample (about 80 ml, or 2 eggcups full). These take about 10 minutes.

We propose to undertake up to two PET scans at Addenbrooke's Hospital in the Wolfson Brain Imaging Centre (WBIC). On each occasion you will be in WBIC for approximately 2 hours, with the scan itself taking 45 minutes in the case of PIB PET and 90 minutes for the others (PK11195 and AV-1451 PET). For each PET scan you will have an injection of about a teaspoon of short lasting radioactive liquid. The radiation dose for each PIB and PK11195 PET scan (2.7 – 3.0 milliSieverts) is similar to the radiation dose we each experience from radiation in the environment during one year living in the East Anglia region, while the radiation dose from AV-1451 PET is 9.3 milliSieverts, which is similar to 3½ years' environmental exposure here. The injected radioactivity fades away naturally over a few hours and you can leave the WBIC as soon as the scan is finished

In all cases our staff would communicate with you throughout the scan to check that you stay comfortable. You could end the scan at any point.

The MRI brain scan will take around an hour and also takes place at the WBIC. This is to look at the size, shape and 'wiring' of the brain. It may be possible to arrange for this to take place on the same day as one of the PET scans. Though MRI scanning is generally very safe, there are certain circumstances where it must be avoided. We will go through a checklist to ask about metal objects attached to or inside your body (e.g. stents, shrapnel, plated fractures,) or electronic devices (e.g. heart pace-maker). Many such items (most modern cardiac stents, for instance) are designed to be MRI safe. Being scanned requires you to lie still and relaxed on a bed in the scanner's 'tunnel'. This 'tunnel' is quite narrow so please let us know if you have experienced claustrophobia in small spaces. It can be noisy but earplugs are supplied and you can also have your own choice of music played over headphones if you wish. As with the PET scans, the technician performing the MRI scan would communicate with you

throughout the scan to check that you stay comfortable. It can be stopped at any point, but takes up to one hour to complete.

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What are the possible benefits of taking part?

This is not a trial of any drug or other treatment and there is no direct benefit to you from taking part in this study. However if you do take part you will be making a significant contribution to medical knowledge and the challenge of dementia especially.

Expenses

If you take part in this study, we would cover all necessary travel expenses and if it would help we would arrange transport by taxi for you to come to the hospital and go home.

Will my taking part in the study be kept confidential?

If you do take part in the study, all information provided to us and the results of studies would be treated confidentially. It will be stored securely in locked cabinets or on password protected computer systems, under the supervision of the Chief investigators. We will retain the data for over 10 years. We will ask for your permission to share your data and scans in an anonymised way with collaborators, now and in the future, including researchers in the NHS, Medical Research Council, University and National Institute for Health Research. The NHS is trying to improve the quality of clinical and research standards. This is being achieved through 'clinical governance'. As part of this process, this study may be reviewed by a clinical governance team. Such a team would need to look at our records to make sure that the research was carried out in accordance with proper procedures.

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The research team should contact you in the next week or so. If, at any time, you need to get in touch with someone, you can contact us:

Professor John T O'Brien (Chief Investigator)	Dr James B Rowe (co-Chief Investigator)
Department of Psychiatry,	Department of Clinical Neurosciences,
University of Cambridge,	University of Cambridge,
Level E4, Box 189,	Herchel Smith Building,

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Summary information sheet for patients

Neuroimaging of Inflammation in MemoRy and Other Disorders (NIMROD)

You are invited to take part in a research study. Taking part is entirely voluntary, in other words, you do not have to take part if you do not want to. This sheet tells you about the study. We will also talk about the study with you and your relative or carer

This is a short summary of the study. Full information is given in the longer information sheets which we have given to your relative/friend/advocate to read. We will also ask their advice about whether or not you want to take part in this study. If you take part, or say no, your normal GP and hospital care will be the same.

As we get older, our memory can let us down. We can forget where things are or what things are called. We can lose interest in things. This can be caused by dementia or depression, which are due to changes in the brain.

The changes in the brain may include inflammation, as well as changes in the size of the brain or chemicals in the brain. We want to know more about how these things cause memory problems, and how they change over time. We can do this using brain scans. Special brain scans can show signs of inflammation for example. We will see how these scans match the memory and other thinking difficulties that people have.

We are looking for volunteers for this study. Some will be in good health, and others will have very mild memory problems. We also want to include people with dementia such as Alzheimer's disease, Lewy body dementia, frontotemporal dementia or vascular dementia.

The volunteers would come to Addenbrooke's Hospital. A researcher (nurse or doctor) would ask some questions about memory, mood and general health, and there would be a brief examination to look at movements and walking. This would take up to two hours. We would like to ask your relative/carers some questions as well. We would ask people to have some tests of memory, language, attention and vision. This can be done at home if you prefer. It will take up to 2 hours and people can take breaks if they get tired.

If you take part, you would have an MRI brain scan and up to two PET brain scans (done on different days). Most patients will have had a scan in the NHS clinics, and these are similar. Each PET scan involves an injection into your arm of a radioactive liquid. The radioactivity fades away over a few hours and you can go home straight after the scan. The amount of radiation dose from each PET scan equates to between one and three and a half years of exposure from natural background radiation by living in the UK. For the PET scans you would lie in the scanner for between three-quarters of an hour and one and a half hours. The MRI scan would last about an hour. The PET scanner is quiet, but the MRI scanner is a quite noisy so we give you earplugs (and you can listen to your own choice of music). Some people find the MRI scanner a little claustrophobic as well so do let us know if this might be a problem. We can hear you and see you all the time and if you want to stop you can let us know at any time. The MRI scan will be looked at by a specialist. Sometimes we find something unexpected on a scan. If this occurs we would let you and your GP know. We may also ask to take a blood sample from you, like an ordinary blood test.

The research team will contact you in the next week or so. If, at any time, you need to get in touch with someone, you can contact:

Professor John T O'Brien (Chief Investigator)

Department of Psychiatry,

University of Cambridge,

Level E4, Box 189,

Addenbrooke's Hospital,

Hills Road,

Cambridge, CB2 0SP

Tel: +44 (0)1223 760682

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Department of Clinical Neurosciences,

University of Cambridge,

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Health Research Authority

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04 June 2013

Professor John T. O'Brien
Foundation Professor of Old Age Psychiatry
University of Cambridge
Department of Psychiatry
Level E4, Box 189, Addenbrooke's Hospital
Cambridge
CB2 0QQ

Dear Professor O'Brien

Study title:	Neuroimaging of inflammation in memory and other disorders (NIMROD). A study of the role of brain inflammation in dementia, depression and other neurological illnesses.
REC reference:	13/EE/0104
IRAS project ID:	117275

Thank you for your letter of 29 May 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Miss Jessica Parfremment, NRESCommittee.EastofEngland-CambridgeCentral@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Appendix 2

Appendix 2 provides additional details relevant to chapter 3, in particular the findings from the post-mortem examination of the proband's father.

Macroscopic observations

Following removal at autopsy, the brain was fixed in 15% neutral buffered formalin, and weighed 1420g. The cerebellum and brainstem were separated from the cerebrum. Examination of the external surfaces found moderate cerebral atrophy, which was most prominent in the frontal and temporal lobes. The left temporal lobe was more severely affected than the right temporal lobe. The atrophy extended to involve the precentral gyrus and the superior parietal gyrus. The cerebral hemispheres were sectioned in the coronal plane. The cerebral atrophy observed externally was confirmed and there was enlargement of the lateral ventricles. The basal ganglia and thalami were unremarkable although the subthalamic nucleus was slightly reduced in size. Horizontal sections of the brainstem revealed pallor of the substantia nigra and locus coeruleus. Apart from a small single infarct in the cerebellar cortex, the cerebellum was unremarkable.

Tissue preparation and immunohistochemistry

Standard tissue blocks from the superior frontal, precentral, inferior temporal and anterior cingulate cortices, the hippocampus at the level of the lateral geniculate nucleus, the basal ganglia at the level of the head of the caudate nucleus, the midbrain at the level of the red nucleus, the pons at the level of the locus coeruleus, subthalamic nucleus, the medulla at the level of the hypoglossal nucleus, and the cerebellar dentate gyrus were embedded in paraffin wax and cut on a rotary microtome. Ten μm sections were stained using haematoxylin and eosin and modified Bielschowsky silver. Immunoperoxidase staining using phosphorylated tau (clone AT8; mouse; 1:1000; Cat. No. MN1012; Thermo Scientific Australia, Scoresby, Victoria) was performed using a Discovery DX autostainer (Ventana Medical Systems, Tuscon, AZ, USA). 3-repeat tau (mouse; 1:50; Cat. No. 05-803; Abcam; Melbourne, Victoria), 4-repeat tau (mouse; 1:50; Cat. No. 05-804; Abcam), p62 (rabbit; 1:250; Cat. No. 610833; BD Biosciences; North Ryde, NSW) and α -synuclein (mouse; 1:500; Cat. No. 610787; BD Biosciences; North Ryde, NSW) were performed manually. For antigen retrieval, 3-repeat tau and p62 required heating in the pressure cooker at 110°C for half an hour in TE buffer (pH 9.0), 4-repeat tau required pre-treatment with formic acid for 15 minutes followed by heating in the pressure cooker at 110°C for half an hour in TE buffer, and α -synuclein required pre-treatment with citric acid buffer (pH 6.0) for 18 minutes in the microwave.

Following blocking of endogenous peroxidase activity in 100% methanol with 3% hydrogen peroxide for ten minutes, sections were blocked in 10% normal horse serum (NHS) in TBS buffer (pH 7.4). Primary antibodies diluted in TBS with 1% NHS were incubated at 37°C for one hour, followed by incubation in EnVision Dual Link Polymer (Cat. No. K4061; DAKO; North Sydney, New South Wales). Dark brown staining was visualised by adding hydrogen peroxidase to a 3'3'-diaminobenzidine solution. All sections were counter-stained with haematoxylin.

Microscopic observations

Histopathology

Sections of the cerebral cortex showed mild neuronal loss and gliosis in the superior frontal and inferior temporal cortices with the normal laminar distribution of neurons preserved. Ballooned neurons were observed in cortical sections stained with haematoxylin and eosin. Both the hippocampal CA1 region and entorhinal cortex showed mild neuronal loss and sparse neurofibrillary tangles. Neuritic plaques were not observed. Consistent with the macroscopic examination, there was mild neuronal loss of pigmented neurons in the substantia nigra and depigmentation in both the substantia nigra and locus coeruleus. The basal ganglia and cerebellum had minimal neuronal loss.

Tau immunohistochemistry

Phosphorylated tau-immunopositive ballooned neurons (Fig 1A), astrocytic plaques, widespread grey and white matter thread pathology (Fig 1B) and coiled bodies (Fig 1C) were observed in the superior frontal and inferior temporal cortices. The morphology and distribution of these inclusions appeared similar to sporadic frontotemporal lobar degeneration cases with CBD pathological subtype (Cairns et al., 2007; D. W. Dickson et al., 2002). Immunostaining with 4-repeat tau and p62 labelled a similar number of ballooned neurons, astrocytic plaques and coiled bodies. Both 4-repeat tau and p62 only labelled a small proportion of phosphorylated tau-immunopositive threads in both grey and white matter. 3-repeat tau-immunoreactivity was not observed. Phosphorylated tau-immunopositive Pick bodies and tufted astrocytes were not observed. Immunostaining with α -synuclein was not observed in the cortex, hippocampus or brainstem. The severity of neuropathological features immunostained with phosphorylated tau was similar in both the superior frontal and inferior temporal cortices with a mild number of ballooned neurons and astrocytic plaques, and moderate to severe coiled bodies and thread pathology in grey and white matter of both regions.